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A STUDY OF METHODS OF ESTIMATION OF METABOLIC NITROGEN

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INTRODUCTION

The so-called metabolic nitrogen of the feces is that portion which has an origin other than as an undigested food residue. It consists of residues from the bile and digestive juices, of epithelium and mucus from the digestive tract, and of such products of bacterial activity as have been derived from digested or from digestible nitrogen.

Our reason for wishing to estimate this fraction of the nitrogen of the feces is that it is a factor which must be considered in the determination of the digestibility of protein—a matter of great importance in relation to practical animal and human nutrition.

The plan of this experiment was to feed a basal ration of corn alone to each of five pigs during the first period, and to add to this corn ration in subsequent periods nitrogenous supplements to be used in the comparison of methods. In the selection of these supplements it was our object to choose foods the protein of which would probably be entirely digestible. Those used were milk, blood albumen, and commercial dried egg albumen.

In the comparison of methods of metabolic-nitrogen estimation it was our object to determine which procedure would yield results representing these assumedly entirely digestible protein foods as being entirely digestible—that is, assuming the proteins of milk, for instance, to be entirely digestible, we made an effort to determine which method of estimation of metabolic nitrogen would assign to the protein of milk a digestion coefficient nearest to 100 per cent.

An experimental study involving so much assumption can not yield results of the highest value, but it was our hope that it might assist in the establishment of a useful conventional procedure.

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The methods of metabolic-nitrogen estimation compared in this study were the acid-pepsin method, the acid-pepsin and alkaline-pancreatin method, and the alcohol, ether, hot-water, and cold-lime-water method suggested in 1888 by Jordan.[1]

The philosophy of the two methods first mentioned is that by the use of digestive enzyms the nitrogen which has been digested, absorbed, and returned to the feces may be separated from the indigestible nitrogen. In using either of these methods we assume that there is no further digestion, during the course of the estimation, of that part of the food protein which escaped digestion in the alimentary tract of the experimental subject. We have no means of proving the truth of this assumption.

The acid-pepsin method represents stomach digestion alone. The acid-pepsin and alkaline-pancreatin method more nearly follows the physiological process, in that intestinal digestion is also represented. The latter method naturally yields decidedly higher results.

In the Jordan method the treatment with solvents is designed especially for the purpose of washing out bile residues, protein cleavage products and mucin.

The exact procedures followed in the three methods are as follows:

ACID-PEPSIN METHOD

Weigh out 5-gm. samples of fresh feces from a weighing bottle; roll up in 9 cm. filter papers, and transfer to 200-c. c. volumeteric flasks. Add 100 c. c. of pepsin-hydrochloric-acid solution (made by adding 1.25 gm. of pepsin to each liter of 0.33 per cent hydrochloric-acid solution). Shake thoroughly and put into an air bath maintained at 38° to 40° C. Allow the digestion to continue for 24 hours. During the first 6 hours agitate by rotation once each hour; agitate again 1 hour before final removal from the air bath. Arrange funnels with 12.5 cm. fluted quantitative papers, and dry 100-c. c. volumetric flasks. Promptly at 24 hours from the time of starting the digestion remove the 200-c. c. flasks from the oven, cool, fill to the mark with cold distilled water, mix thoroughly, and filter. Determine the nitrogen in 100 c. c. of the filtrate. The result represents metabolic nitrogen.

ACID-PEPSIN AND ALKALINE-PANCREATIN METHOD

Weigh 1.5 to 2.5 gm. samples of fresh feces into 150 c. c. Jena beakers. Add 100 c. c. of acid-pepsin solution (1.25 gm. of pepsin to each liter of 0.33 per cent hydrochloric acid). Stir thoroughly with a glass rod and place in an air bath maintained at 38° to 40° C. Stir thoroughly once each hour for the first 8 hours. Allow the digestion to continue for exactly 24 hours. Filter immediately through 12.5 cm. fluted quanti-

[1] [Jordan, W. H.] Analytical and experimental methods. Protein digestion. *In* Maine Agr. Exp. Sta. Ann. Rpt. 1888, p. 197. 1889.

tative filters. Wash beakers, filters, and contents until free from acid, with water at a temperature of 40° C.

Return filters and contents to the proper beakers and treat with 100 c. c. of alkaline-pancreatin solution (1.5 gm. pancreatin in somewhat less than 1 liter of water; add 3 gm. of sodium carbonate (Na_2CO_3); dilute to exactly 1 liter, and mix thoroughly). Return the beakers to the bath and stir thoroughly. Allow to digest for exactly 12 hours. Filter immediately through fluted papers. Wash beakers, filters, and contents thoroughly and repeatedly with hot water, and allow to dry. Transfer the filters and contents to Kjeldahl flasks and determine the nitrogen in the usual manner. Subtract the result from total nitrogen of the feces; the remainder represents metabolic nitrogen.

JORDAN'S METHOD[1]

Weigh 2 to 3 gm. portions of fresh feces, and dry at 100° to 105° C.; transfer to extraction capsules and extract with ether for 16 hours. Transfer to 150-c. c. beakers and treat with 50 c. c. of boiling 95 per cent alcohol. Keep at boiling temperature for 10 minutes; decant the alcoholic extract through qualitative filters; wash several times with hot alcohol and once or twice with ether, by decantation. With a camel's-hair brush transfer the residue from the filter papers to the original beakers; add 50 c. c. of hot water and boil for 10 minutes; filter through the same papers used for the last filtration, washing with hot water, by decantation. Wash the residues from the filter papers back into the beakers with 50 c. c. of a saturated solution of calcium hydrate, and let stand for 6 hours; filter through the same filters last used; transfer all the material from the beakers to the filter papers; wash with lime water, and allow to drain. Transfer filter papers and contents to Kjeldahl flasks, and determine the nitrogen. Subtract result from total nitrogen of the feces; the remainder represents metabolic nitrogen.

EXPERIMENTAL PROCEDURE

The subjects of this experiment were five Yorkshire barrows of nearly uniform age and weight. The average weight at the end of the first period was 53.85 kgm., and at the end of the fourth, 59 days later, 84.42 kgm., the average daily gain in weight being 518 gm., or 1.14 pounds. They were confined in the metabolism crates illustrated in our previous publications.[2]

[1] **Jordan, W. H.,** Op. cit. (Detailed specifications were not submitted in the original publication; the particulars as here stated were arbitrarily assumed.)

[2] **Forbes, E. B., Beegle, F. M., and others.** A chemical study of the nutrition of swine. Ohio Agr. Exp. Sta. Bul. 271, p. 224–261, 3 pl. 1914.

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The experimental periods were of 10 days' duration, separated by 7-day intervals during which were fed the rations of the periods to follow. The feces were marked with carmine.

Table I records the total amounts and nitrogen content of the foods consumed and feces produced.

Table II records the percentages of total nitrogen in the feces and of metabolic nitrogen, as estimated by the three different methods.

Table III records the coefficients of digestibility of the nitrogen of the foods.

TABLE I.—*Foods consumed and total nitrogen in foods and feces*

Period No. (10 days).	Pig No.	Foods consumed.		Nitrogen in foods.		Weight of feces.	Total nitrogen of feces.	
		Corn.	Supplements.	Corn.	Supplements.			
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent.*	*Gm.*
I	1	18,200	.....	245.700	.....	6,357	0.995	63.252
	2	19,731	.....	266.373	.....	7,553	.889	67.146
	3	20,000	.....	270.000	.....	7,954	.989	78.665
	4	18,000	.....	243.000	.....	5,866	1.005	58.953
	5	16,800	.....	226.800	.....	5,645	1.000	56.450
			Blood albumen.		Blood albumen.			
II	1	16,200	730	215.298	83.987	5,737	.888	50.945
	2	18,000	810	239.220	93.191	6,144	.785	48.230
	3	19,000	855	252.510	98.368	6,710	1.006	67.503
	4	17,100	770	227.259	88.589	5,428	.892	48.418
	5	17,100	770	227.259	88.589	5,226	.943	49.281
			Skim milk.		Skim milk.			
III	1	17,100	21,400	230.679	111.708	5,857	1.082	63.373
	2	19,000	23,800	256.310	124.236	6,566	.900	59.094
	3	19,000	23,800	256.310	124.236	6,936	1.059	73.452
	4	17,100	21,400	230.679	111.708	5,672	1.066	60.464
	5	17,100	21,400	230.679	111.708	5,529	1.060	58.607
			Egg albumen.		Egg albumen.			
IV	1	17,100	770	234.441	87.226	5,129	1.226	62.882
	2	19,000	855	260.490	96.854	5,847	.989	57.827
	3	19,000	855	260.490	96.854	6,263	1.346	84.300
	4	17,100	770	234.441	87.226	5,410	1.177	63.676
	5	17,100	770	234.441	87.226	5,371	1.070	57.470

TABLE II.—*Total and metabolic nitrogen of feces (per cent)*

Periods (10 days).	Pig No.	Total nitrogen.	Metabolic nitrogen.		
			Pepsin-hydrochloric acid method.	Pepsin-pancreatin method.	Jordan's method.
I	1	0.995	0.706	0.836	0.473
	2	.889	.619	.760	.418
	3	.989	.701	.834	.468
	4	1.005	.656	.816	.447
	5	1.000	.741	.858	.445
II	1	.888	.585	.749	.422
	2	.785	.581	.677	.404
	3	1.006	.773	.857	.446
	4	.892	.641	.738	.368
	5	.943	.712	.820	.380
III	1	1.082	.714	.831	.426
	2	.900	.590	.693	.347
	3	1.059	.739	.857	.392
	4	1.066	.704	.814	.416
	5	1.060	.691	.859	.420
IV	1	1.226	.802	.965	.525
	2	.989	.670	.761	.451
	3	1.346	.935	1.123	.599
	4	1.177	.729	.949	.565
	5	1.070	.742	.840	.433

TABLE III.—*Coefficients of digestibility of nitrogen*

Period No. (10 days).	Pig No.	Apparent digestibility *a*	Pepsin-hydrochloric acid method.	Pepsin-pancreatin method.	Jordan's method.
I (corn)	1	74.26	92.52	95.89	86.49
	2	74.79	92.34	96.34	86.64
	3	70.86	91.52	95.43	84.65
	4	75.74	91.58	95.44	86.53
	5	75.11	93.55	96.47	86.19
II (blood albumen)	1	105.33	98.48	101.04	102.80
	2	112.96	106.22	102.27	109.18
	3	106.18	105.87	101.57	101.20
	4	107.58	106.22	102.26	102.45
	5	108.22	102.92	101.80	102.21
III (skim milk)	1	96.42	96.15	95.33	93.50
	2	104.44	99.42	96.61	98.34
	3	100.00	99.63	98.15	94.43
	4	95.97	99.01	96.62	94.81
	5	98.93	95.96	97.34	96.84
IV (egg albumen)	1	97.09	95.17	95.70	95.09
	2	108.10	101.34	96.08	103.45
	3	91.33	96.23	97.87	92.98
	4	92.20	94.84	98.12	98.25
	5	101.01	97.14	95.33	97.89

a On basis of total nitrogen of the feces.

CONCLUSIONS

The apparent digestibility of the protein of corn, based on the total nitrogen of the feces is about 75 per cent. On account of the existence in the feces of nitrogen of metabolic origin we know that the real digestibility is higher. The acid-pepsin method makes it appear that the real digestibility of the protein of corn is about 92 per cent, and the pepsin-pancreatin method about 96 per cent. Jordan's method gives appreciably lower figures, averaging 86 per cent.

The acid-pepsin method indicates that 70 per cent, the pepsin-pancreatin method 84 per cent, and the Jordan method 46 per cent of the nitrogen of the feces from corn is of metabolic origin.

All of the methods make the nitrogen of blood albumen appear more than completely digestible, even the apparent digestibility being over 100 per cent; thus, the feeding of blood albumen with corn seems to increase the digestibility of the corn protein to an extent more than sufficient to offset the incompleteness of digestibility of the protein of this supplement.

With skim milk the apparent digestibility varies from 95.97 to 104.44 per cent, the average being 99.15. With the acid-pepsin method three out of the five figures average 99.35. In previous work [1] five estimations by this method averaged 99.12. With the pepsin-pancreatin method the results were lower than with the acid-pepsin method. These low results on the supplementary food are reciprocals of the high results on the basal ration of corn.

The proteins of skim milk are made to appear more nearly completely digestible by the acid pepsin method than by the pepsin-pancreatin method or by the Jordan method.

With egg albumen the results varied considerably, but all were high. It would appear that raw, commercial, dried egg albumen is almost perfectly digested by swine.

Important inaccuracy seems to be inevitable in any determination of digestibility of supplementary foods in the usual way, by difference; and no other method seems more satisfactory. This applies equally to computations of real digestibility, and of apparent digestibility (based on total nitrogen of the feces).

The digestion coefficients for protein involved in the feeding standards of our reference works on animal production assume that the nitrogen of the feces is entirely an indigestible food residue. The rough measures afforded by the results of this study indicate that, as applying to the digestive capacities of swine, this assumption underestimates the digestibility of protein by about 20 per cent.

By way of interpretation of the individual variations in the digestion coefficients we would record the fact that pig 1, in Period II, manifested

[1] Forbes, E. B., Beegle, F. M., and others. Op. cit.

a pronounced dislike for the blood albumen. The acid-pepsin method indicates that this pig was less able than others to digest this foodstuff. Also, we would observe that, in response to most insistent demands for food, we gave to pig 5 in Period II a larger allowance of food per unit of body weight than was given to the other individuals. The digestion coefficient for blood albumen, as determined by the acid-pepsin method, with this pig also is low. Further, these two pigs, No. 1 and 5, had the lowest digestion coefficients, as determined by the acid-pepsin method, in the following period, No. III, where skim milk was fed.

In a study of the effects on metabolic nitrogen of storage of the feces in a frozen condition for 20 days, with and without the addition of thymol, compared with air-drying the fresh material, with and without thymol, no significant differences were observed which could be related to these methods of preservation.

In attempting to choose between these methods it seems to us that the acid-pepsin and the pepsin-pancreatin methods give results which are more nearly true than does Jordan's method, since the latter does not digest the bacteria, which may contain large proportions of the nitrogen of the feces and which presumably are more largely the product of digestible than of indigestible protein; but it is idle to attempt close comparisons of such conventional and inaccurate procedures. We have no accurate scientific basis for the determination of the digestibility of protein.

A NEW STRAIN OF RHIZOCTONIA SOLANI ON THE POTATO

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INTRODUCTION

Investigators heretofore have spoken of strains of *Rhizoctonia solani* Kühn when referring to cultures isolated from different hosts as well as to different isolations from the same host. Thus, Duggar[2] states (p. 423):

> The potato is the most interesting of the host plants with respect to the parasitism of *Rhizoctonia* by reason of the many types of disease induced under diverse conditions. The conditions may be in part climatic and, in part perhaps, dependent upon the pathogenicity of the particular strain of the fungus . . .

He further states (p. 442):

> Strains do occur, however, evidence of which may persist for some time in the general appearance of the cultures.

Edson[3] says—

> Much confusion exists regarding the identity of the various forms, and there is likewise great diversity of opinion as to the pathogenic properties of the members of the group.

Thus, while it is recognized that differences do exist, it has not been shown that it is possible to distinguish the different strains, especially from cultures obtained from the same host, either from their morphology or their growth on various media. Thus, Peltier[4] makes the following statement:

> . . . hence, on the measurement of the mycelial cells of *Rhizoctonia Solani*, as on the study of the growth on media, no conclusions can be based in regard to the distinguishing strains of this difficult species.

The purpose of this paper is to present evidence that two strains of *R. solani* are found on the potato (*Solanum tuberosum*), and, further, that it is possible to distinguish these with accuracy from the macroscopic growth on various media, as well as by the more accurate morphological comparisons.

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[1] The writers are indebted to Dr. H. A. Edson, of the Office of Cotton, Truck, and Forage-Crop Disease Investigations, for advice and suggestions during the progress of this work.

[2] Duggar, B. M. Rhizoctonia crocorum (Pers.) DC. and R. solani Kühn (Corticium vagum B. & C.), with notes on other species. *In* Ann. Mo. Bot. Gard., v. 2, no. 3, p. 403-458, 9 fig. 1915. Bibliography, p. 452-458.

[3] Edson, H. A. Seedling diseases of sugar beets and their relation to root-rot and crown-rot. *In* Jour. Agr. Research, v. 4, no. 2, p. 151. 1915.

[4] Peltier, G. L. Parasitic Rhizoctonias in America. Ill. Agr. Exp. Sta. Bul. 189, p. 372. 1916.

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SOURCE OF MATERIAL

The cultures of *R. solani* on which the following studies are based were obtained from stems and tubers of potatoes grown in Florida and northern Maine. The isolations were made during the summers of 1915 and 1916, so that the comparisons are made with cultures of comparatively the same age. For the sake of convenience they are here designated "R1," "R2," "R3," etc. R1, R2, and R3 were isolated in Maine during the summer of 1916 from the base of affected plants. R4, R5, and R6 were obtained from the same locality from the inside of potato stems. R7 was isolated from tubers obtained at Hastings, Fla., in 1915, and R8 from tubers in Maine during the summer of 1915. Throughout these studies all the strains, from R1 to R8, could not be distinguished, with the single exception of R5. In presenting the results, therefore, R5 will be compared with a representative of one of the other cultures. The cultures from the stems were obtained from plants showing a girdling and hollowing at or near the surface of the ground (Pl. 25, *A*). This condition appeared to be a secondary stage in a malnutrition trouble briefly described by Edson and Schreiner.[1]

DISTINGUISHING CHARACTERS

The points of difference between R5 and the other strains, as here presented, can be grouped as pathological, as shown by inoculation experiments; physiological, as shown by the reactions on different media; and morphological, as shown by the measurements of mycelium, of surface sclerotial cells, and of diameters of germ tubes produced by germinating sclerotial cells.

PATHOLOGICAL CHARACTERS

Inoculations were made in the field on healthy growing plants. The method of procedure was to wash any dirt from the stems, make a slight incision with a flamed scalpel, and insert a bit of a young growing culture into the wound. The control plants were likewise injured. A number of such inoculations with R5, R1, R2, R3, etc., and a number of undetermined fungi also isolated from diseased stems, resulted in the production of very pronounced lesions with R5. The lesions produced by R1, R2, etc., were indefinite, as was also the case with all the undetermined fungi. The injured control plants remained healthy. Plate 25, *B*, illustrates the results of one of the series of inoculations. The three stems to the left were inoculated with R5, the next two with R1 and R2, and the last shows the condition of the control.

Inoculations were also repeated in the greenhouse, with results practically similar to those described above.

[1] Edson, H. A., and Schreiner, Oswald. A malnutrition disease of the Irish potato and its control. In Phytopathology, v. 7, no. 1, p. 70–71. 1917.

Inoculations on the tubers were also made. The method followed was to select clean and healthy tubers, immerse these for 10 minutes in a 1 to 1,000 mercuric-chlorid solution, wash with sterilized water, make a slight incision with a sterile scalpel, and insert a bit of a pure culture in the wound. Control tubers were treated in a similar manner, the agar medium without the fungus being inserted. The lesions produced with any of the strains were never very large, but a very distinct lesion was produced with R_5 as compared with the other strain. Plate 26, *B*, illustrates a tuber inoculated with R_5. For comparison a control tuber is shown in Plate 26, *C*. In several instances the lesion as a result of the inoculation extended to ¼ inch in diameter.

The strain R_5, therefore, is not only more pathogenic on the stems than the remaining strains isolated from the potato, but is in fact able to produce a distinct necrosis of the tissues of the potato tuber.

PHYSIOLOGICAL CHARACTERS

The various strains were grown on a variety of media. Only those on which R_5 has shown any marked distinguishing characteristics will be pointed out here.

POTATO AGAR.—On this medium, when grown in test tubes, R_5 at the end of a week or 10 days produces a very marked discoloration of the medium. This coloration is dark brown, approaching black. The discoloration, if produced at all by the other strains on this medium, is very much less pronounced and never approaches the intensity of color produced by R_5.

CORN-MEAL AGAR.—On this medium, when grown in test tubes, R_5 produces light-gray, loosely formed sclerotia as compared with the darker, brownish, and more compact sclerotia formed by the other strains (Pl. 26, *A*). The character is very striking and can be relied upon.

USCHINSKY'S SOLUTION.—One hundred cubic centimeters of this solution was poured into 200-c. c. Erlenmeyer flasks and inoculated with small bits of pure cultures of the various strains grown on potato agar. The rate of growth of R_5 is far in excess of the remaining strains. At the end of 10 days R_5 entirely covered the surface and was growing on the side of the flask, while the growth of the remaining cultures were still below the surface of the liquid.

MORPHOLOGICAL CHARACTERS

In holding up against the light some petri-dish cultures on string-bean agar of the various strains, the writers were struck with an apparent difference in the fineness of the mycelial strands of R_5 as compared with the remaining strains. A mount made and examined under the microscope revealed a distinct difference in the fineness of the mycelium. This difference in the diameter of R_5 and the remaining strains was very evident

microscopically without definite measurements. Subsequent examinations made with the microscope, from cultures of various ages, as well as from a variety of different media, showed this difference constant, the diameter of the mycelium of R_5 being in every case smaller than that of the remaining strains. Measurements of R_5 and the remaining strains were made from cultures of the same age grown on the same media. The procedure followed was to grow the cultures in petri dishes, and mounts on slides were made a definite distance from the original planting. Fifty measurements were made at random in each case. The results of these measurements show that the diameter of R_5 varies from 4.7 to 8.8 μ, with 7.8 μ as the average measurement, while the measurements of the remaining strains vary from 10 to 14.0 μ, with 10.1 μ as the average. Figure 1 illustrates this difference comparatively, both sections being drawn with the aid of the camera lucida, with the same magnification and from cultures of the same age. That the thickness of the mycelium is a good distinguishing character was confirmed as follows: 19 mounts were made from cultures grown on various media and of varying ages. The R_5 was marked at the time of mounting, but in such a way that the person to whom they might be submitted was unaware of any distinguishing marks. These were submitted to two pathologists of the Office of Cotton, Truck, and Forage-Crop Disease Investigations, with a request that they divide the mounts into two lots, based on the diameter of the mycelium. In every case the mounts of R_5 were separated from the remaining strains.

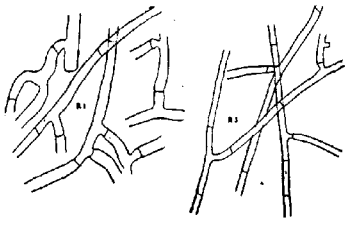


FIG. 1.—Camera-lucida drawings of mycelium of the R_1 and R_5 strains from the same medium and cultures of the same age.

Not only is the diameter of the mycelium of R_5 smaller, but likewise the short sclerotial cells enveloping the sclerotia are smaller, as shown in figure 2. One hundred measurements show that those of R_5 vary in length from 13.6 to 30.6 μ, with an average of 21.6 μ, while the others measure 17 to 61.2 μ, with an average of 37.5 μ. In width those of R_5 measure from 8.3 to 20.4 μ, with an average of 12.3 μ. The other strains measure from 11.9 to 23.3 μ, with an average of 16.7 μ. Generally they are also much more regular than those found in the remaining strains, so much so, in fact, that they can be described as "monilia-like." In the case of the others, R_1, R_2, etc., while occasionally one sees a chain of regular sclerotial cells, this is the exception rather than the rule. It may perhaps be argued that R_5 was contaminated with another sterile fungus the mycelium of which resembles that of a species of Rhizocotonia, but is of

a smaller diameter. It was not possible to obtain a culture from a single basidiospore, but dilution plates were made and a culture obtained from the germination of a single short typical sclerotial cell. This culture

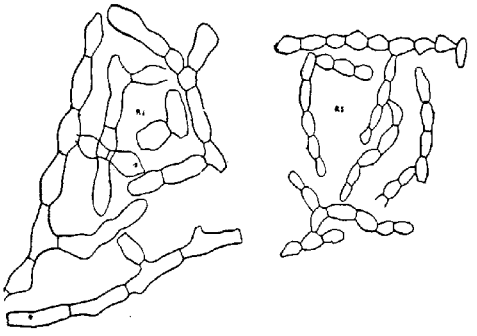


FIG. 2.—Camera-lucida drawings of short sclerotial cells of the R1 and R5 strains from cultures of the same age grown on the same medium.

agreed in all essential characters previously enumerated with the other cultures of R5.

While the mycelium measurements of R5 and all the remaining strains with which the writers worked are distinct, they do show variation, as indicated by the measurements given above. There may also be a question as to the accuracy of random measuring, even from mounts made from the same medium of the same age. A more accurate manner of identification of R5 from the remaining strains consists in the germination of the short sclerotial cells in water and the measurement of the diameter of the germ tubes produced. The mode of germination of the short sclerotial cells of R5 agreed in all essential characters with that of the remaining strains and has been described by Duggar[1] and others. Measurements made of the diameter of the germ tubes produced by R5 and the remaining strains

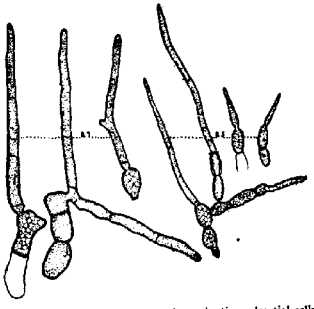


FIG. 3.—Camera-lucida drawings of germinating sclerotial cells of the R7 and R5 strains. The drawing was made at the end of 17 hours, after the cells were placed to germinate in drops of water.

[1] Duggar, B. M. Op. cit.

showed that the germ tubes produced by R_5 are constantly smaller than those of the remaining strains. Figure 3 illustrates this relation. The writers consider this character the most reliable in the identification of these strains. In making the measurements of the germ tubes the question arose whether different length germ tubes produce tubes of varying width and also whether the size of the sclerotial cell is correlated with the size of the germ tube. In order to determine this point, 100 measurements were made of the short sclerotial cells, the length of the germ tube, together with the width of same. The results may be summarized as follows: The size of the cell has no apparent effect on the length or width of the germ tube; nor has the length of the germ tube any appreciable effect on the diameter of the germ tube. The width of the germ tubes produced by the germinating enveloping sclerotial cells of R_5 vary from 3.4 to 6.8 μ, with an average of 4.3 μ, while those of R_7 and R_4, taken as representatives of the remaining strains, vary from 6.8 to 11.9 μ, with an average of 8.5 μ.

Table I summarizes the measurements made in the above comparative studies.

TABLE I.—*Comparative measurements of strains R5 and R7 of Rhizoctonia solani*

	Diameter of mycelium.		Sclerotial cells.				Diameter of germ tubes.	
			Length.		Width.			
	R_5	R_7	R_5	R_7	R_5	R_7	R_5	R_7
	μ	μ	μ	μ	μ	μ	μ	μ
Minimum	4.7	10.0	13.6	17.0	8.3	11.9	3.4	6.8
Maximum	8.8	14.0	30.6	61.2	20.4	23.3	6.8	11.9
Average	7.8	10.1	21.6	37.5	12.3	16.7	4.3	8.5

COMPARISON WITH RHIZOCTONIA CROCORUM (Pers.) DC.—Duggar gives the mycelial measurements of *R. crocorum* (Pers.) DC. as 4 to 8 μ; of *R. solani* Kühn, 8 to 12 μ. According to the measurements of the writers, R_5 varies from 4.7 to 8.8 μ and the common strain of *R. solani* from 10 to 14 μ. These measurements agree so closely with those of *R. crocorum* and *R. solani* as given by Duggar that there appeared to be need for a closer comparison with *R. crocorum*. Such a comparison was made with exsiccatæ material, with material of *R. crocorum* on asparagus collected in Germany by Dr. H. A. Edson, of the Office of Cotton, Truck, and Forage-Crop Disease Investigations, and kindly furnished by him to the writers, and lastly with the descriptions and distinguishing characters as outlined by Duggar.[1] Unfortunately, the original specimens from which the isolations were made were not saved, as at that time it was not known that the strain of Rhizoctonia isolated was essentially different

[1] Duggar, B. M. Op. cit.

Hybrids of Zea ramosa and Zea tunicata

PLATE 17

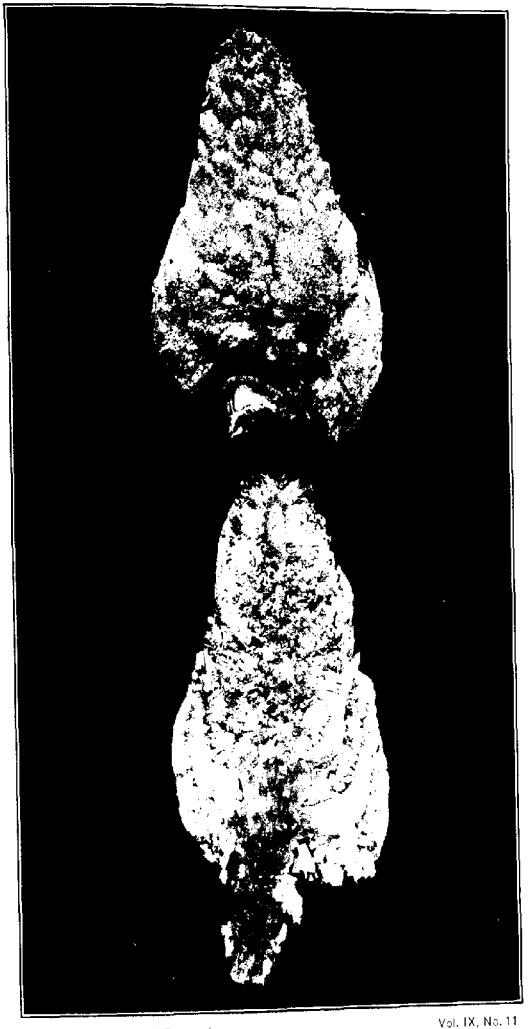
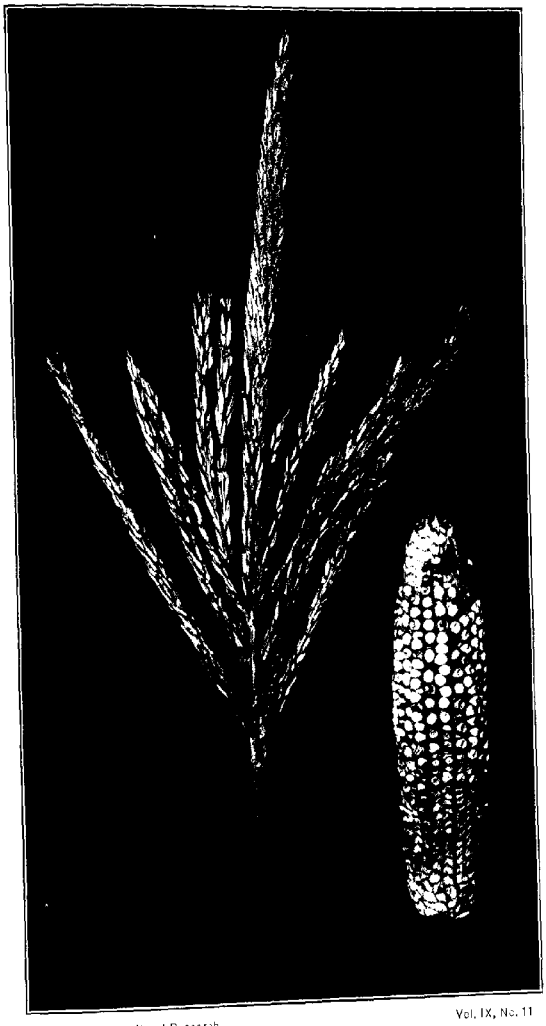


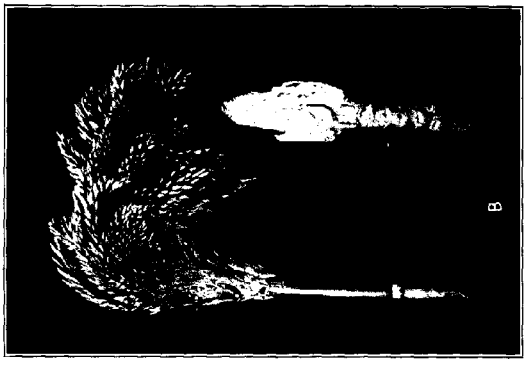
PLATE 18

Terminal inflorescence, cauliflower type of F_2 plant of *Zea ramosa* × *Zea tunicata* hybrid.

PLATE 19

Half-tunicate F_2 plant, the nearest approach found to an intermediate between normal and half tunicate.




B

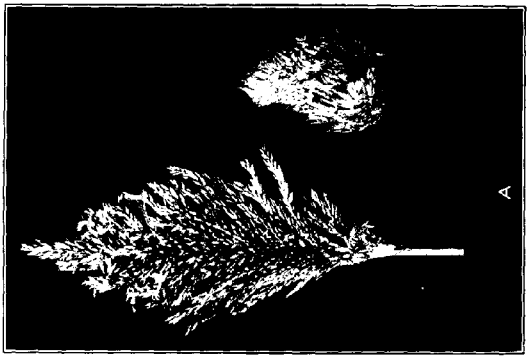

A

PLATE 20

A.—Terminal and lateral inflorescence of F_2 plant, showing the ear both branched and tunicate, and the tassel with only *ramosa* characters.

B.—Terminal and lateral inflorescence of F_2 plant, combining both *ramosa* and *tunicata* characters.

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PLATE 21

Pistillate inflorescence of F_2 plant, showing both the branched and tunicate characters.

EUPATORIUM AGERATOIDES, THE CAUSE OF TREMBLES[1]

By R. S. CURTIS, *Animal Husbandman, Beef-Cattle and Sheep Investigations*, and FREDERICK A. WOLF, *Botanist, North Carolina Agricultural Experiment Station*[2]

INTRODUCTION

In the mountainous sections of North Carolina considerable losses of domestic animals annually occur from a malady known as trembles. This disease is said to be transmissible to man through the ingestion of milk, certain milk products, or flesh of animals affected with trembles, and is known by physicians as milksickness. Since trembles is of so much economic importance to the live-stock interests of the State and since the investigations dealing with its etiology contain such diverse conclusions, a study of this disease was begun during 1916. It is deemed advisable to present at this time the considerable body of data which has accumulated relative to the relationship between *Eupatorium ageratoides* (Pl. 22) and trembles and to reserve for future investigation many important considerations which are as yet unknown or lack conclusive data. Data on certain other phases of the problem have already been secured, but a report of this part of the investigation is reserved for future publication.

HISTORICAL REVIEW

A very large number of papers dealing with milksickness, or trembles, have appeared in the hundred or more years during which the disease has been known. Most of these articles have been published in the various medical journals, and references to the most important of them are given in several of the more recent investigations (1, 2, 3, 4, 5, 6).[3] These recent papers, furthermore, contain a brief résumé of the important findings of the earlier students of this disease, so that it is necessary for the present purpose to review only the pertinent facts relative to the causes which have been regarded as productive of trembles. In general, these etiological factors may be placed in one of three groups—namely, mineral poisons, poisonous plants, and bacterial parasites.

Arsenic, lead, and cobalt are among the minerals which have been suspected of being the cause of the trouble, but these charges have been disproved.

Among the poisonous plants whose ingestion is regarded as the cause of the trouble are *Rhus toxicodendron*, *Eupatorium ageratoides*, *Lobelia inflata*, and *Bigelovia* (*Isocoma*) *heterophylla*. Moseley (4) was of the

[1] Published with the permission of the Director of the North Carolina Agricultural Experiment Station.

[2] Grateful acknowledgment is hereby made of the kindly assistance in this work of our colleagues Mr. Earl Hostetler and Dr. J. I. Handley.

[3] Reference is made by number to "Literature cited," p. 404.

opinion, from experiments in which *E. ageratoides* was fed to various animals, that this weed was the cause of the disorder. His results were not entirely convincing, however, and in criticism of them Crawford (1, p. 15) says:

> It can not be said that Moseley has even proved *Eupatorium ageratoides* to be a poisonous plant, much less the cause of "trembles."

When, in the summer of 1906, about 50 head of cattle died of trembles near Minooka, Ill., an investigation of *E. ageratoides* was undertaken by the Office of Poisonous Plant Investigations of the United States Department of Agriculture, since it was the popular belief that this weed was the cause of the trouble. Aqueous extracts were prepared from dried plants and plants preserved in water to which a small amount of chloroform had been added. These extracts were fed or injected subcutaneously into rabbits, cats, and dogs. An aqueous extract from the ash of dried plants was also administered to rabbits. Fifty-eight gm. of fresh plants were, furthermore, fed to a lamb weighing 25 kgm. without the production of symptoms of trembles. In summarizing the results of these experiments, Crawford says (1, p. 19–20):

> It certainly can not be said that it has been proved that milksickness is due to any constituent of *Eupatorium ageratoides*. * * *. Again severe epidemics have occurred in winter when the foliage has disappeared, which would tend to exclude the higher, nonevergreen plants as the cause of this disorder. In fact, all the evidence in hand is against the causation of this disease by such plants.

Subsequent publications by Moseley (5, 6) advance the claim that aluminium phosphate found to be present in leaves and stems of *E. ageratoides* is the active toxic principle. Animals fed on this weed or on food in which aluminium phosphate was mixed were found to void aluminium phosphate in the milk and urine. The blood of these animals and certain organs were, moreover, found to contain aluminium phosphate. The stems of rayless goldenrod (*Isocoma heterophylla*) were also found to contain aluminium phosphate; and when this weed, too, was fed to rabbits it produced symptoms similar to those following the feeding of *E. ageratoides* or aluminium phosphate. Since aluminium phosphate is insoluble in water, this accounts, as explained by Moseley, for Crawford's failure to produce poisoning in the experiments in which aqueous extracts of *E. ageratoides* were used. His criticism of the experiment in which 58 gm. of fresh weed were fed to a lamb weighing 25 kgm. is that this quantity would probably not be fatal to a full-grown rabbit.

Experiments conducted by Jordan and Harris (2, 3) in New Mexico and Texas, where this disease is present, but where *E. ageratoides* does not grow, indicate that the disease is of bacterial origin. Trembles developed in rabbits, guinea pigs, dogs, and calves by inoculation with a spore-forming organism which the authors described as *Bacillus lactimorbi*. This organism was present in the milk and butter of cows affected with trembles, in the feces of nonfatal cases in man, in certain parts of the

bodies of affected sheep and horses, and in the soil in regions where milksickness prevails. Taken as a whole, however, the experiments were far from decisive in showing that *B. lactimorbi* is the etiological factor in the production of trembles, or milksickness.

METHODS OF EXPERIMENTATION

Since *E. ageratoides*, commonly called "white snakeroot," does not grow in the vicinity of Raleigh, N. C., where the experiments were conducted, it was arranged to secure daily shipments of the green weed from Shooting Creek, N. C., where *E. ageratoides*, locally known as "richweed," grows luxuriantly, especially in shady situations. Since this place is about 300 miles distant from Raleigh, the weed used had been cut about 48 hours prior to its arrival at Raleigh. The weed was fed twice daily to sheep kept singly in small pens in a sheep barn. A maintenance ration of some dry concentrate was given in addition to this green food. The animals used were selected from the experimental flock of grade ewes, all of which were in a healthy condition and, with the exception of those used in the experiments, remained so. The flock number of each individual was retained and is used subsequently in reporting the experiments with the several animals. No case of trembles had ever appeared in this or any other of the Station flocks prior to or during the course of these experiments.

Each animal was weighed when it was placed on the experiment and at time of death. Beginning with experiment 3, in addition to the initial weighings subsequent weighings were made at 3-day intervals until the experiment was concluded or until death resulted. At first the grain and weed were fed separately; but, since the animals either avoided eating any of the weed or ate only sparingly of it, the weed was passed through an ensilage cutter and then mixed with grain before being fed. A daily account was kept of all of the food which was refused by each animal, and these data were employed in approximating the total amount consumed during the course of the experiment. The quantity of *E. ageratoides* eaten by each animal could only be approximated, since the weed and grain refused were mixed and since some loss of weight was due to desiccation. Post-mortem examinations were made of ewes 10, 11, 14, 27, 161, and 169. With the exception of ewe 169, the post-mortem examination showed no evidence that death resulted from any other cause than the feeding of *E. ageratoides*. Post-mortem examinations were not made of the other animals, because the external symptoms were clearly those which characterize trembles.

SYMPTOMS OF TREMBLES

Since the possibility exists that trembles in animals may develop from causes other than the feeding of *E. ageratoides* and that the symptoms may differ somewhat from those resulting from the ingestion of this

weed, a brief account of the symptoms observed in the experiments with sheep is pertinent. Considerable individual variation exists in the different animals, both in the period elapsing until the first symptom of trembles is apparent and in the period following until death ensues. Some were sick as early as three days after being placed on the experiment, and no effects were apparent for three weeks in other cases. Sheep usually live three or four days after the disorder is first noticed. Some remain alive, however, for nearly two weeks, and one animal characteristically affected entirely recovered. The feeding of *E. ageratoides* to this animal, however, was discontinued as soon as trembling was noted.

Sheep in the early stages of the disease are sluggish and lie quiet unless urged to rise. They may refuse to eat, or the appetite may be quite normal. There is generally a very considerable decrease in weight, most of which occurs in the last two or three days preceding death. Respirations are accelerated and somewhat labored. A marked stiffness of the legs and ataxia characterizes the movements in walking. If after a day or two the animal is made to stand for a few minutes or is driven a few yards, muscular spasm, especially in the limbs, is evident. The sheep then stands with hind limbs placed well under the body (Pl. 23, *A*) and all feet spread apart laterally. In this posture the back is bowed, the neck outstretched, and the head lowered. Within a few seconds the quivering spreads over the entire body, increases in intensity, and becomes a violent, involitional tremor (Pl. 23, *B*). This is accompanied by slight, intermittent, tetanic contractions of the musculature of the limbs. At this stage of trembling ataxia is very pronounced, and the animal is unable to stand (Pl. 24, *A*). It drops quickly into the normal resting posture (Pl. 24, *B*), whereupon the trembling immediately ceases. If the sheep is made to rise after it has lain down for a few moments, a second spasm of trembling ensues, with a repetition of the symptoms as described. Trembling may recur repeatedly every time the animal is made to stand. The quiescent period is shortened, however, after each spasm of trembling and may begin as soon as the animal is placed on its feet.

RESULTS OF EXPERIMENTS

EXPERIMENT 1.—Three ewes, No. 11, 26, and 10, were used in experiment 1, which was designed to determine whether harmful effects follow the feeding of *E. ageratoides*. This experiment was begun on June 17 and closed on August 2. However, from June 22 to July 6 and from July 18 to July 28 it was impossible to secure the weed. During these periods the animals were grazed on Bermuda grass pasture. Except during the two periods mentioned, a liberal supply of white snakeroot was fed just as it arrived from the point of shipment. In addition, a maintenance ration of grain was fed in a separate trough. Neither the weeds

nor the grain were weighed in this experiment. Initial and final weights of each animal were recorded.

In the period between June 17 and July 16 ewe 11 was fed on *E. ageratoides* and grain for an aggregate of 15 days. A typical case of trembles had developed by July 16, and death occurred two days later. Food was refused during these two days, and there was a decrease in weight from 102 to 77 pounds during the 29 days of intermittent feeding.

Ewe 26 was given a ration of snakeroot and grain for 22 days, between June 17 and August 2. No symptoms of trembles developed during this period. The initial weight of this animal was 91 pounds, and the weight at the time the experiment was discontinued was 74 pounds.

The control ewe, No. 10, was maintained on pasture alone from June 17 to July 28. On July 28 she was put in a pen and was given a ration of *E. ageratoides* and grain until her death, which occurred on August 2. This ewe trembled only slightly, was very weak and emaciated, and lost 20 pounds during the experiment. Although the symptoms were not as marked in this case as in ewe 11, yet all conditions indicated that death was due to trembles.

EXPERIMENT 2.—Three ewes, No. 14, 23, and 26, were employed in experiment 2. This experiment was planned to confirm the results secured in experiment 1. Since the animals used in experiment 1 had refused to eat any considerable quantity of snakeroot when it was fed separately, it was decided to pass the weed through an ensilage cutter and mix it with an equal quantity by weight of grain. One pound of this mixed feed was given each animal twice daily.

On the sixth day after ewe 14 was placed on the experiment she had developed trembles and died on the following day. Her initial weight was 80 pounds, and her weight at death was 73 pounds.

The first symptom of trembles in the case of ewe 23 was noted 19 days after the experiment was begun. A well-defined case of trembles developed in this animal, and she died 6 days after the first symptoms were noticed. Her weight when feeding was begun was 70 pounds, and there was a loss in weight of 8 pounds during the 25 days.

Since ewe 26 had shown no ill effects from the feeding of *E. ageratoides* in experiment 1, she was used in this experiment. It will be recalled that the weed was fed separately and was not ground in the first experiment. Ewe 26 had eaten only sparingly of the weed in this experiment. However, after 16 days' feeding with the mixed ration a very typical case of trembles developed. The feeding of the weed was therefore discontinued and she was put on pasture.

EXPERIMENT 3.—In this experiment ewes 12, 7, 29, 27, and 19 were fed the mixed ration to determine the amount of weed and the length of time required to develop trembles. Table I shows clearly the variation that exists with reference to these two points.

TABLE I.—*Results of feeding Eupatorium ageratoides to sheep—Experiment 3*

Ewe No.	Initial weight.	Experiment begun—	Feeding discontinued—	Days before death occurred.	Weight at death.	Feed consumed.	
						Grain.	Weed.
	Pounds.				*Pounds.*	*Pounds.*	*Pounds.*
12	74	Aug. 5	Aug. 18	[a]13	.....	5.5	11.5
7	86	...do.....	Sept. 1	27	61	13	9
29	81	...do.....	Aug. 21	16	55	8	10
27	70	...do.....	Aug. 10	5	56	2.25	4.5
19	113	...do.....	Aug. 23	18	89	8	11.25

[a] Feeding discontinued after 13 days.

Ewe 12 was taken off the experiment on August 18, at which time she was affected with trembles. She had lost only 1 pound during these 13 days. It will be noted that the amount of weed required to cause trembles in these five animals varied from 4½ to 11½ pounds and the range in time from 5 to 27 days.

EXPERIMENT 4.—In order to determine whether or not trembles is infectious, ewes 26 and 12 were put in a small Bermuda grass lot on August 18. Two healthy ewes from the flock were put in the same lot and all were fed grain in the same trough. It will be recalled that both ewe 26 and ewe 12 had typical cases of trembles when their feeding in experiments 2 and 3, respectively, was discontinued. Ewe 26 died on August 19 and ewe 12 still trembled a week afterwards. However, she finally recovered fully. Neither of the other two ewes had developed any symptom of trembles when the experiment was discontinued on September 4, and both subsequently remained normal.

EXPERIMENT 5.—This experiment was designed to determine the length of time that *E. ageratoides* must be fed to sheep when, after a certain number of days, the usual grain ration and pasturage are given. Two animals were therefore fed for three days on a mixture of equal parts of ground weed and grain and were then put on pasture. Two others were fed for six days before being placed on pasture and two others for nine days, after which they were put on pasture. Table II contains the essential facts in this experiment.

TABLE II.—*Results of feeding Eupatorium ageratoides to sheep—Experiment 5*

Ewe No.	Initial weight.	Final weight.	Days on experiment.	Feed consumed.	
				Grain.	Weed.
	Pounds.	*Pounds.*		*Pounds.*	*Pounds.*
169	83	78	3	1.5	1.5
171	85	80	3	1.5	1.5
162	96	82	6	5.5	5.5
168	89	79	6	4.25	4.25
161	102	62	9	6.75	6.75
170	105	92	9	5.5	5.5

Ill effects followed only in the cases of ewes 169 and 161, the former dying 8 days and the latter 11 days after being taken off the experiment. Since ewe 169 evidenced no marked symptoms of trembles, a post-mortem examination was made which showed that stomach worms (*Hemonchus contortus*) may have been a contributory cause of her death. Ewe 161, however, developed a typical case of trembles and is the animal represented in the accompanying illustrations (Pl. 23, 24).

EXPERIMENT 6.—Since certain sodium compounds have been suggested as antidotes for trembles, two sheep were given definite quantities of common stock salt and one baking soda along with the mixed ration of *E. ageratoides* and grain. This experiment was conducted between September 18 and October 20. The data on these three animals are presented in Table III.

TABLE III.—*Results of feeding Eupatorium ageratoides; together with salt or soda, to sheep.—Experiment 6*

Ewe No.	Initial weight.	Placed on feed—	Date of death.	Days before death resulted.	Weight at death.	Feed consumed.	
						Grain.	Weed.
	Pounds.				*Pounds.*	*Pounds.*	*Pounds.*
21.....	121	Sept. 18	Sept. 29	11	.....	8	5.25
28.....	96	...do.....	Oct. 7	19	73.5	15	10.25
37.....	118	Oct. 2	Oct. 20	18	78.5	10	6

Ewe 21 consumed 8 ounces of salt and ewe 37 ate 12 ounces during the periods of 11 and 18 days, respectively, in which they were on the experiment. Ewe 28 ate with her feed 30 ounces of baking soda.

EXPERIMENT 7.—Since it has been claimed that aluminium phosphate causes a disorder similar to that following the feeding of white snakeroot, two ewes, 166 and 167, were fed aluminium phosphate for a period extending from September 9 to November 17. During this time each ewe was fed 412 gm. of aluminium phosphate ($AlPO_4$; Baker's, C. P.) mixed with 68.5 pounds of grain, this being supplemented with 138 pounds of alfalfa hay. The daily amounts of aluminium phosphate given were gradually increased from 2 to 16 gm.

At no time during this period of 69 days were these ewes observed to manifest any symptoms of trembles. The initial weight of ewe 166 was 80 pounds, and her weight at the close of the experiment was 91 pounds. The initial and final weights of ewe 167 were 90 and 93 pounds, respectively.

SUMMARY

(1) *Eupatorium ageratoides*, commonly known as white snakeroot and locally known in North Carolina as richweed, had previously been claimed by Moseley to cause trembles in animals. This claim has been substantiated by experiments with sheep in which green plants of *E.*

ageratoides were fed during the months of June, July, August, September, and October, 1916.

(2) Fifteen cases of trembles in sheep have been developed from feeding *E. ageratoides*. Fourteen of these resulted fatally, and one of them recovered. Death of one of these sheep was probably due in part to an infestation of stomach worms.

(3) Death resulted in from 5 to 27 days following the beginning of feeding of *E. ageratoides*.

(4) Considerable variation existed in the several ewes, also, with reference to the quantity of weed ingested before trembles appeared.

(5) Indirect evidence against the infectious nature of the disease was secured by failure to communicate trembles from sheep characteristically affected to healthy sheep when they were confined and fed together in a small lot.

(6) Salt and soda in the amounts given along with a ration of grain and *E. ageratoides* were without apparent antidotal effect.

(7) No harmful effect followed the feeding for 69 days of aluminium phosphate mixed with grain and supplemented with alfalfa hay.

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PLATE 22

Eupatorium ageratoides, or white snakeroot.

PLATE 25

A.—Potato stems, showing the nature of the lesions from which isolations of *Rhizoctonia solani* were made.

B.—Potato stems, showing the results of one series of inoculations. Three stems to the left were inoculated with strain R_5, the next two inoculated with R_1 and R_2, and the stem to the extreme right an injured control. The inoculations were made on August 14, 1916, and the photograph was taken on September 4, 1916.

from the common strain of *R. solani*. It is therefore impossible to state with any degree of accuracy the color of the mycelium and sclerotia on the host, the presence or absence of infection cushions, etc. The writers, however, are reasonably sure that these characters were not those belonging to *R. crocorum*. Aside from the above consideration, its similarity to *R. crocorum* is shown by (1) the close agreement of mycelial measurements, (2) the sclerotia in culture approaching the plectenchymatic type. The differences are shown by (1) the myceliums lacking the typical violet or violet brown pigment in cultures of considerable age on a variety of media, (2) the branchings not being at right angles, though occasionally so, and (3) most important perhaps, the ease with which it is grown in culture on a variety of media contrary to the experience of all former investigators.

A consideration of the above facts lead the writers to believe that R_5 is to be regarded as a distinct strain of the common *R. solani* Kühn as occurring on the potato rather than one of *R. crocorum* (Pers.) DC.

SUMMARY

A strain of *R. solani* Kühn, for the sake of convenience temporarily designated as "R_5," was isolated from potato stems in Maine during the summer of 1916. This strain can be distinguished from the more common *R. solani* by (1) the more pronounced lesions produced when inoculated on injured stems or tubers; (2) the reaction, growth, and character of sclerotia on definite media; and (3) morphologically, by measurements of the mycelium, of the short sclerotial cells, and, lastly, by the measurement of the diameter of germ tubes when the short, or "barrel-shaped," cells enveloping the sclerotia are placed in drops of water to germinate.

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stopper inside of the flask, and the compound was allowed to evaporate. After several different insects were used in preliminary tests, the house fly (*Musca domestica* L.) was selected as being typical and easy to breed in large numbers. The flies were bred in the insectary and kept under natural conditions, thus avoiding irregular results due to the different ages and physical conditions of the wild flies. Five flies were put into each flask, the chemical introduced, and the flask tightly stoppered. When all the flies in the flask were apparently dead, they were removed to a vial and given 24 hours to revive. If none revived, the time during which the flies were exposed to the vapor was recorded. But, if the flies revived, the experiment was repeated. The average of 50 tests for a certain quantity of any chemical was found to be practically the same as the average of 5 tests; hence, in each case 5 tests were conducted. Controls showed that flies could live in a closed flask for 20 or more hours.

Since similar weights of the different chemicals do not contain the same number of molecules, and their toxicity could not, therefore, be accurately compared, it was decided to determine the toxicity in minutes for similar fractions of a gram-molecule of each chemical. Different quantities of each chemical were tested and curves plotted. As the quantity increased, it was found that each chemical had a point beyond which an increase would not give a reduction in the time required to kill. This is the point at which the air is saturated with the vapor, and differs for each chemical. As the quantity is decreased, a point is reached where the vapor is not of sufficient strength to kill. The plotted curves lie between these two points.

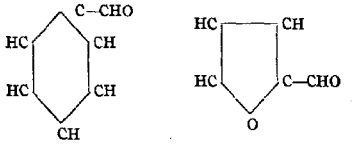
After the curves were plotted, it was found to be impossible to compare similar fractions of a gram-molecule; hence, the different fractions of a gram-molecule necessary to kill in a fixed time of 400 minutes were determined. A long period of time was selected as a more nearly correct index of toxicity. The fraction of the gram-molecule was determined by dividing the amount of the chemical necessary to kill in 400 minutes by the molecular weight of the substance. The sums given in the charts are the millionths of a gram-molecule necessary to kill five house flies in a 1-liter flask at a temperature of 70° F.

The liquid benzene compounds were measured by volume in blood-counting pipettes, and the weight of this volume was determined from the weight of 1 c. c. of the chemical. Weighed quantities of the solid benzene derivatives were dissolved in a known volume of benzene. A certain volume of this solution would contain a definite quantity of the benzene derivative. The measured volume was placed on the paper and blown for a moment to evaporate the solvent. The rapid evaporation of the solvent resulted in a lowering of the temperature, thus preventing appreciable evaporation of the compound to be tested.

RESULTS OF THE INVESTIGATION

Inasmuch as carbon bisulphid is in general use as a fumigant, its toxicity was determined for comparison with the toxicity of the benzene derivatives. Figure 1 shows curves based upon different amounts of certain of the chemicals and the time required to kill with such quantities. Owing to the extended curves of some of the substances a graphical representation was not feasible.

The data upon which the curves are based are given in Table I. Furfural is included for comparison with the aldehydes of benzene. Its relationship to benzenaldehyde is shown in the following formulas:



The results, given in millionths of a gram-molecule, are recorded in Table II and shown graphically in figure 2.

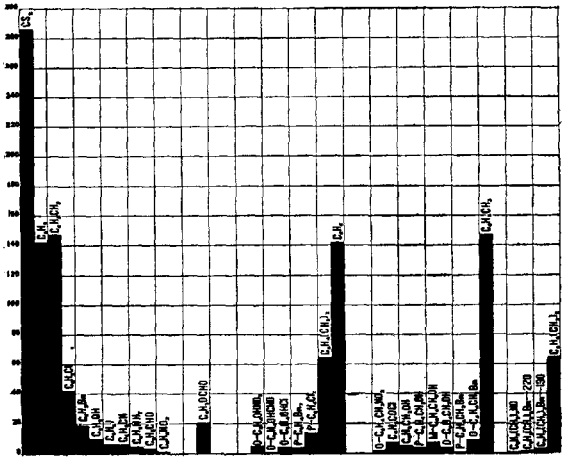


FIG. 2.—Graph showing the millionths of a gram-molecule necessary to produce the death of five house flies in a 1-liter flask at 70° F. in 400 minutes.

TABLE I.—*Quantity of a chemical and time required to kill five house flies in a 1-liter flask at 70° F.*

Chemical.	Quantity of chemical required.	Time required (average of five tests).	Chemical.	Quantity of chemical required.	Time required (average of five tests).
	Gm.	*Minutes.*		*Gm.*	*Minutes.*
Carbon bisulphid	0.02179	400	Salicylic aldehyde	.00044	93
Benzene	.02028	185	Do	.00029	110
Do	.01521	349	Do	.00014	349
Do	.01015	427	Do	.00007	735
Toluene	.01548	258	Ortho-nitrophenol	.001	241
Do	.01289	498	Do	.0007	409
Do	.01032	600	Do	.0005	512
Chlorbenzene	.01265	85	Ortho-bromtoluene	.00265	159
Do	.00632	215	Do	.00177	341
Do	.00413	566	Do	.00132	610
Brombenzene	.00475	133	Para-bromtoluene	.001	318
Do	.0038	160	Do	.0005	350
Do	.0028	461	Do	.00025	351
Do	.0019	646	Do	.0001	544
Phenol	.003	155	Ortho-cresol	.00064	182
Do	.002	213	Do	.00048	333
Do	.001	411	Do	.00042	661
Iodobenzene	.00347	87	Meta-cresol	.00196	223
Do	.00231	123	Do	.00130	304
Do	.00115	576	Do	.00065	479
Benzonitrile	.00190	122	Para-cresol	.00193	249
Do	.00137	166	Do	.00129	319
Do	.00063	427	Do	.00064	360
Anilin	.00128	137	Do	.00032	435
Do	.00064	204	Benzoyl chlorid	.00257	130
Do	.00048	511	Do	.00164	273
Benzaldehyde	.00134	178	Do	.00082	486
Do	.00067	219	Ortho-nitrotoluene	.00058	281
Do	.00033	595	Do	.00036	293
Nitrobenzene	.00152	130	Do	.00029	450
Do	.00076	176	Bromxylene (B. P. 190°-250° C.)	.002	201
Do	.00038	254	Do	.001	292
Do	.00019	451	Do	.0005	487
Xylene	.01005	95	Bromxylene (B. P. 220°-250° C.)	.00087	269
Do	.00754	305	Do	.00043	356
Do	.00502	911	Do	.00022	532
Para-dichlorbenzene	.003	200	Nitroxylene	.00028	368
Do	.002	424	Do	.00014	581
Do	.001	670	Furfural	.00297	155
Para-dibrombenzene	.001	371	Do	.00223	273
Do	.0008	621	Do	.00198	437
Do	.0006	759			
Ortho-chlorphenol	.00112	131			
Do	.00075	181			
Do	.00056	512			

TABLE II.—*Quantity of a chemical necessary to kill five house flies in a 1-liter flask in an arbitrary time of 400 minutes*

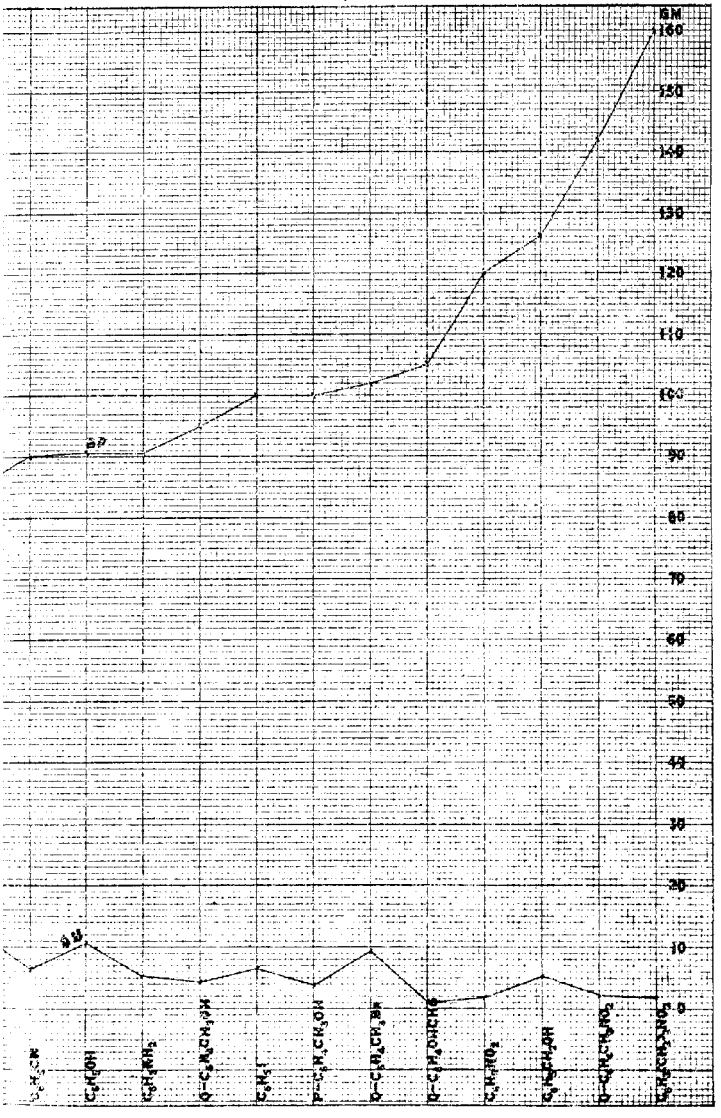
Chemical.	Quantity of chemical required (in millionths of a gram-molecule).	Chemical.	Quantity of chemical required (in millionths of a gram-molecule).
Carbon bisulphid	286. 3	Salicylic aldehyde	1. 1
Benzene	142. 3	Ortho-nitrophenol	5. 6
Toluene	1475	Ortho-bromtoluene	9. 4
Chlorbenzene	42. 4	Para-bromtoluene	1. 2
Brombenzene	19. 2	Ortho-cresol	4. 2
Phenol	10. 8	Meta-cresol	7. 9
Iodobenzene	6. 6	Para-cresol	3. 9
Benzonitrile	6. 4	Benzyl alcohol	5. 3
Anilin	5. 3	Benzoyl chlorid	7. 8
Benzaldehyde	3. 7	Ortho-nitrotoluene	2. 1
Nitrobenzene	1. 8	Bromxylene (B. P. 190°–210° C.)	3. 5
Xylene	6. 4	Bromxylene (B. P. 220°–250° C.)	1. 9
Para-dichlorbenzene	14. 0	Nitroxylene	1. 7
Para-dibrombenzene	4. 1	Furfural	20. 8
Ortho-chlorphenol	4. 6		

DISCUSSION OF RESULTS

TOXICITY AND CHEMICAL COMPOSITION

By a glance at figure 2 it is noticed that all the benzene compounds used are more toxic than carbon bisulphid. The introduction of a methyl group into the benzene ring decreases its toxicity. This result agrees with the findings for higher animals of Winternitz and Hirschfelder (7) and further studies of Kline and Winternitz (3). The introduction of a halogen increases the toxicity similar to the results of Bechhold and Ehrlich (1), who found the introduction of a halogen increased the disinfection properties of phenol. This fact is true for insects whether the halogen is introduced in benzene, toluene, xylene, or phenol.

One might expect that, as toluene is less toxic than benzene, the halogen derivative of toluene would be less toxic than the similar derivative of benzene; but such does not seem to be the case. The iodin derivatives are more toxic than the corresponding bromin compounds, while both are more toxic than the corresponding chlorin derivatives. The disubstitution compounds of the halogens are more toxic than the monosubstitutions. The introduction of the cyanogen group does not increase the toxicity as much as might be supposed. The aldehyde group greatly increases the toxicity; in fact, salicylic aldehyde is the most toxic of all the compounds used in the experiments. From this result it would be expected that furfural would be much more poisonous than the results show it to be. Substitutions in the methyl group of toluene are



...nt (*BP*) of the compound and its toxicity expressed in milliontls of a gram molecule (*GM*).

less toxic than in the benzene ring. Para configurations seem to be more toxic than ortho configurations, while the only meta derivative tried was less toxic than either. Although certain relationships exist between chemical composition and toxicity, they are not as striking or as constant as might be expected.

BOILING POINTS AND TOXICITY

In working over the results, the author noticed a relationship between the boiling point of the chemical and its toxicity. As many of the compounds bore no guaranty of purity, the boiling points of several were determined and a curve plotted, showing the chemicals in order from the lowest boiling point to the highest. In comparison, a curve of toxicity of these compounds was plotted, as shown in figure 3. The curves show strikingly that the higher the boiling point the more toxic is the chemical. Exceptions are to be noted, which may be due to the rôle played by chemical composition in either raising or lowering the toxicity; but, in general, the curve is an increase of toxicity with an increase in the boiling point. Benzaldehyde shows a break in the curve, possibly owing to a specific action of the aldehyde. The low boiling point of furfural (96° C.) may account for its toxicity being less than would be expected from its chemical composition. Carbon bisulphid, having the lowest boiling point (47° C.), lower than any of the benzene derivatives tested, is likewise the least toxic of all the compounds.

The explanation of the relationship of boiling point to toxicity has not been ascertained. Whether the introduction of a certain element or group causes an increase in toxicity incidental to an increase in the boiling point or whether it is the relationship of boiling point to vapor pressure and volatility is not known.

BOILING POINT AND LIPOID SOLUBILITY

Another interesting observation is the relationship between boiling point and lipoid solubility. To test the lipoid solubility of the compounds, cephalin was extracted from the brain of an ox by Hirschfelder's method (2).

> Ox brain was covered with three volumes of alcohol, shaken up two or three times, and the excess of alcohol then poured off and squeezed out gently through linen, care being taken to avoid great force in wringing out the alcohol, as this tends to break up the brain tissue into very finely divided particles which pass through the filter. The residue is then covered with three volumes of ether, shaken vigorously, and filtered first through cotton and then through filter paper. The clear filtrate thus obtained is evaporated to dryness over a water bath and a yellow residue remains.

The cephalin so prepared was placed in capsule heads of 0.08 c. c. capacity and introduced into 1 c. c. of the chemical to be tested. It was found that benzene boiling at 78.5° C., toluene at 107.5° C., and xylene at 130° C. dissolved several capsules of cephalin until it finally

became thick and pastelike. By placing cephalin under a small bell jar, the air of which remained saturated with benzene, it absorbed benzene from the air until finally a liquid mass was produced. The same was true for toluene and xylene. This shows that cephalin and either benzene, toluene, or xylene are miscible in all proportions. On the other hand, brombenzene, with a boiling point of 150° C., dissolved but one capsule in 1 c. c. Benzaldehyde (165° C.) slowly penetrated the cephalin, but dissolved but little of it; while anilin (170.5° C.) salicylic aldehyde (185° C.), nitrobenzene (200° C.), and nitroxylene (240° C.) did not penetrate the cephalin and dissolved but very slight traces of it. Five c. c. of nitrobenzene, evaporated to dryness, left a very slight greasy mark on the evaporating dish. An effort was made to extract cephalin from the brain tissue with nitrobenzene without success. Lanolin also is practically insoluble in nitrobenzene. One c. c. of benzene containing 0.16 c. c. of cephalin was poured into 10 c. c. of nitrobenzene and the mixture blown with an electric fan until the benzene was evaporated, resulting in the cephalin's being thrown out of solution. From these results it appears that compounds with high boiling points are poor lipoid solvents, but are the most toxic to insects. These experiments would indicate that an increase in lipoid solubility as determined by the above method causes a decrease in toxicity in the chemicals used. Further work is now in progress to determine whether a similar relationship exists between the boiling point, lipoid solubility, and toxicity of a wider range of chemicals from the alipathic series and the terpenes.

TOXICITY OF BENZENE DERIVATIVES TO OTHER INSECTS

The toxicity of the benzene derivatives was found to be similar for other insects, and although this work has not been completed, one point may be noted. A comparison of the bluebottle fly (*Lucilia sericata* Mg.) with the house fly (*Musca domestica* L.) shows that house flies die more quickly from compounds with a low boiling point than bluebottle flies, while compounds with a high boiling point are more toxic to the bluebottle flies than to the house fly. Similarly, the cockroach (*Blatella germanica* Linn.) succumbs less readily than the potato beetle (*Leptinotarsa decemlineata* Say) to low boiling compounds and more readily to high boiling compounds. This relationship may be due to morphological differences in the insects, possibly the diameter of the spiracles or trachea.

CONCLUSIONS

Although no effort has yet been made to apply the results, certain possibilities are apparent. Even if the compounds with low boiling points are less toxic than those with high boiling points, inasmuch as more of such compounds may be evaporated before saturation is reached, better results may be obtained. This is shown in figure 4, which gives the maximum amount (in pounds) that will evaporate in 1,000 cubic feet of

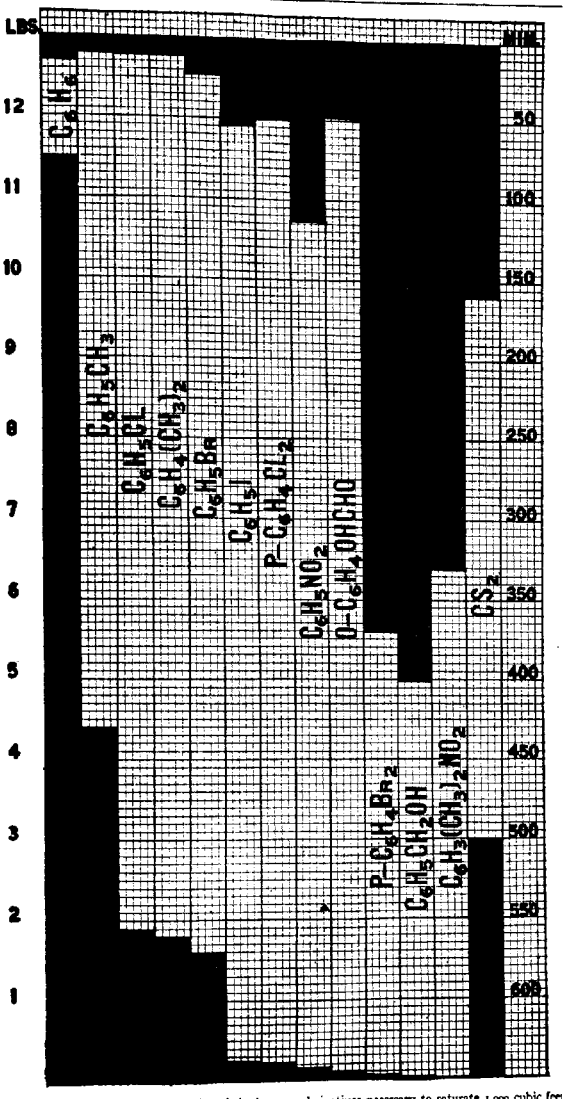


FIG. 4.—Graph showing the quantity of the benzene derivatives necessary to saturate 1,000 cubic feet of space at 70° F. and the time required by such quantity to kill house flies. Carbon bisulphid at the standard rate is given for comparison.

space at 70° F., and the time required for such quantity to kill house flies. Carbon bisulphid at the rate of 3 pounds to 1,000 cubic feet is compared with the benzene derivatives. As a low-boiling compound will penetrate grain better than a high-boiling compound, the possibilities of xylene, chlorbenzene, and brombenzene are at once apparent. Tests of the value of these compounds in the fumigation of grain have not been made. Inasmuch as the vapor of many of the benzene compounds is explosive when mixed with air, one must observe certain precautions, although in general they are far less explosive than carbon bisulphid.

For the fumigation of animals a compound with a high boiling point is needed in order that relatively little of the material shall be in the air to be taken in by the animal or to irritate the eyes or nose. In this respect salicylic aldehyde is probably the best. The cost of this chemical is prohibitive for general fumigation; but, inasmuch as higher animals readily oxidize it to salicylic acid, which is very slightly poisonous, this compound might be used for the internal fumigation of horses to destroy bots as carbon bisulphid is now used. As previously stated, it has been decided to try out a large series of chemicals before selecting the best compounds for tests as to their practicable possibilities.

SUMMARY

Data are presented showing the toxicity of certain organic compounds, mainly from the aromatic series, to insects, particularly the house fly, and certain general relationships are indicated.

(1) All the benzene derivatives tested proved to be more toxic to insects, molecule for molecule, than carbon bisulphid.

(2) Physical characters, such as boiling point and vapor pressure, have more influence on the toxicity than chemical composition.

(3) Up to 250° C. the higher the boiling point the more toxic the compound to insects. Beyond 250° C. the compound is usually so slightly volatile that not enough of the chemical will evaporate to be effective.

(4) Lipoids are very soluble in compounds with low boiling points and but slightly soluble in compounds with high boiling points.

(5) Compounds with low boiling points, although less toxic, owing to their great volatility, may give better results than compounds with high boiling points, particularly in the fumigation of grain.

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HYBRIDS OF ZEA RAMOSA AND ZEA TUNICATA

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INTRODUCTION

Data regarding the domestication of maize are still extremely meager. Yet a knowledge of this important agricultural step would be of such importance in tracing the early history of man and the beginnings of civilization that any investigations promising to throw light on the subject need no further justification.

The present study deals with the behavior of a hybrid between the two most striking variations or mutations from normal maize. Both have been considered as distinct species, *Zea tunicata* and *Zea ramosa*. Though usually referred to as agricultural species, they seem to deserve a place with the so-called species of Oenothera, which have originated through mutation.

DESCRIPTION OF PARENTS

Zea tunicata, or "pod corn," is a rather well-known variation of ordinary maize (Pl. 13, 14, 15). The most striking characteristic is that the glumes of the female inflorescence, or ear, are developed so that each seed is entirely inclosed. Associated with this character is a less conspicuous lengthening of the glumes of the staminate inflorescence that results in a thickening of the tassel (Pl. 13, *B*).

The origin of *Z. tunicata* is not known, but its occurrence in widely separated and isolated regions would indicate that it has originated independently more than once, presumably as a mutation from ordinary maize. So far as known, it has never appeared in pedigreed cultures, but there is at least one instance where it is reported to have appeared in a carefully bred commercial variety (Sconce, 1912).[1]

Z. tunicata is reported from Paraguay, Brazil, Argentina, Belgian Kongo, and many places in the United States. It was known to many tribes of American Indians. According to Parker (1910), both the Senecas and Mohawks had special names for tunicate maize, that in Seneca being translated as "original corn."

Thus far we have found no definite reference to tunicate maize in Mexico, the reference given by Sturtevant (1894) being obviously a misidentification. Neither has it been reported from Peru, and in a most extensive vocabulary of the Quichua terms relating to maize obtained in Peru by Mr. O. F. Cook, of the Bureau of Plant Industry, who made

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 395.

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special inquiry for this type of maize among well-informed people familiar with Indian agriculture, there is no mention of tunicate maize.

In hybrids with nontunicate varieties the tunicate character behaves as a dominant, but in our experiments we have never been able to secure a homozygous tunicate strain. Progenies resulting from the selfing of tunicate plants have, with us, always shown segregation into approximately three tunicate plants to one normal.[1]

The tunicate plants in self-pollinated progenies are separable into two classes, one producing typical tunicate ears and thickened tassels like the parent plant (Pl. 13, *B;* 14, *B*), the other with greatly enlarged tassels containing both staminate and pistillate flowers, and with the ear either aborted or bearing greatly enlarged and usually sterile spikelets (Pl. 13, *A;* 14, *B;* 15). This last class represents approximately one-third of the tunicate plants. Although these plants produce what appears to be normal pollen in the terminal inflorescence, the long glumes never open and the pollen is not shed; and we have not been successful in securing selfed seed of this form.

The ratios in which the different classes occur would indicate that the class with the bisexual terminal inflorescence is the homozygous form and that the ordinary tunicate plants represent the heterozygous form, a cross between the form with the bisexual inflorescence and the normal nontunicate maize. If the ordinary tunicate type can occur in a homozygous form, we should expect one in four of the plants grown from a self-pollinated tunicate plant to be homozygous, and the progeny of such homozygous plants should be all podded, whether cross or self-pollinated. This has not been the case in our experiments. If only a few progenies were grown, the failure to secure an all-tunicate progeny might, of course, be ascribed to the accidental selection of heterozygous instead of homozygous parents.

In the course of our experiments the progenies of 43 different tunicate plants have been grown and in all of these progenies, except one, nontunicate plants appeared. The one exception produced only eight plants. And, since only two or three normal plants were expected, it is not surprising that none appeared. Of the remaining 42 parent plants, 14 might have been expected to prove homozygous. That none proved to be homozygous can hardly be accidental, since the chances against it are over 400,000 to 1. It is therefore concluded that, in the material that has come under our observation, the ordinary type of tunicate plants represents a case of imperfect dominance, and that it is unfixable,

[1] A comparison of the ratios of tunicate to nontunicate plants shows the following:

	Tunicate.	Nontunicate.
Self and tunicate×tunicate	288	94
Expected	286.5±5.7	95.5
Tunicate×nontunicate	99	117
Expected	108 ±5.0	108

like the Andalusian fowls. Our experiments have contained tunicate strains from three distinct sources; but, since other workers report the existence of pure-tunicate strains, it may be that still other stocks behave differently.

The distinction between full tunicate and half tunicate has not always been made in our pedigrees, but the records show 17 progenies where the number of full-tunicate plants is recorded. The total number of plants in these 17 progenies is 187, of which 46 were classed as full tunicate. If, as suggested, the full-tunicate plants are the homozygous form, the expected for the number of individuals involved would be 62, a deviation of 16, or four times the probable error, a rather large deviation to be ascribed to chance, but not sufficiently aberrant to offset the failure to secure homozygous individuals among the half-tunicate plants. The distinction between full and half tunicate is not always easy to make, and it would appear from the ratios that we have been referring some of the less pronounced examples of the full tunicate to the half-tunicate class. By concentrating selection on this group of plants, more or less intermediate between full and half tunicate, it may be possible to secure a homozygous strain. But in the stocks with which we have been experimenting, individuals of the type shown in Plate 13, *A*, or that shown by East and Hayes (1911) would not serve as examples of pure-tunicate maize.

The class with bisexual terminal inflorescence, which is here assumed to be homozygous, will be referred to as "full tunicate" and the ordinary tunicate type, which we look upon as heterozygous, will be termed "half tunicate." The term "tunicate," or podded, will be used as a general term including both of the above classes.

Z. ramosa, or branched maize, is a variation from ordinary maize discovered by Dr. W. B. Gernert (1912) at the Illinois Agricultural Experiment Station. The original ear was found in 1909 in a field of Leaming corn.

Z. ramosa differs from the normal maize in having the pistillate inflorescence, or ear, which is normally simple, replaced by a compound inflorescence branched like the tassel (Pl. 14, *A*, *c*). There is also a less striking but equally significant change in the branching of the tassel (Pl. 16). In normal maize the terminal inflorescence bears a number of branches at its base. Above the uppermost branch the axis is continued into what is termed the "central spike," where the pairs of spikelets are borne directly on the main axis of the inflorescence. Thus, in passing from the base to the tip of the tassel, there is an abrupt transition from the uppermost branch to simple pairs of spikelets. In the *Z. ramosa* tassel the branches are much more numerous and gradually decrease in size from the base upward, the transition from branches to pairs of spikelets being imperceptibly gradual.

Unlike *Z. tunicata*, *Z. ramosa* is a recessive variation. The dominance of normal maize over this variation seems complete. We have never been able in any way to distinguish between plants heterozygous for the *ramosa* character and normal maize. So far as observed, the character behaves as a simple Mendelian unit.

Krafft (1870) described and figured a branch pistillate inflorescence that appears to have been of the same character as *Z. ramosa*. The reference is of interest as indicating that *Z. ramosa*, as well as *Z. tunicata*, may be looked upon as having originated more than once.

NATURE OF THE VARIATIONS

In normal maize one of the characters differentiating the male and female inflorescence is that the glumes are greatly reduced in the pistillate inflorescence, so that the kernels are naked on the cob. In *Z. tunicata* this differentiation is lost, and the kernels are inclosed in glumes as long as those of the staminate inflorescence.

Another specialized character of normal varieties of maize is the suppression of the branches in the pistillate inflorescence, or ear. In *Z. ramosa* this specialization is lost, and the ear is as completely branched as the tassel. A further loss of specialization in *Z. ramosa* is that the differentiation between branches and pairs of spikelets is lost, the one grading into the other in both ear and tassel.

In considering these losses of specialization it is desirable to keep in mind the fact that in *Z. tunicata*, as in ordinary maize, the ear is a pistillate homologue of the central spike of the tassel, while in *Z. ramosa* the ear is a pistillate replica of the entire tassel. The loss of specialization in *Z. tunicata* affects the characters of the floral organs and spikelets, while in *Z. ramosa* the general form of the pistillate inflorescence is changed to conform to that of the tassel.

Both *Z. ramosa* and *Z. tunicata* are variations from normal maize toward the general type of grasses, and as such may be looked upon as reversions, since both cases involve a loss of a specialization that distinguishes maize from practically all other grasses.

Recent investigations have shown that many reversions may be explained as recombinations, but neither *Z. ramosa* nor *Z. tunicata* results from the recombination of latent factors, or cryptomeres. Had the first appearance of *Z. ramosa* and *Z. tunicata* been the result of the fortuitous combination of factors, or cryptomeres, it would seem to follow that when the new combination was crossed with the parent stock these factors should have been again redistributed and the combination necessary to bring the new characters again into expression should have occurred much less frequently than the observed one in four individuals.

Both of these variations may also be considered either as examples of homœosis (Leavitt, 1909) or as metaphanic variations (Cook, 1915).

The transference of long glumes and the branched conditions from tassel to ear might be looked upon as excellent examples of homœosis. There is, furthermore, a large series of abnormalities that occur in the terminal inflorescence of full-tunicate plants that lend themselves to this interpretation. Spikelets develop into branches, glumes develop into pistils, lodicules develop into glumes, and so on.

On the other hand, it is difficult to think of the nicely graded length of the branches of the tassel of *Z. ramosa* as a translocation of characters. This character is better explained as a metaphanic variation or loss of differentiation, an explanation that would also apply to the branched ear of *Z. ramosa* and the lengthened glumes on the ears of *Z. tunicata*. But here the variation rather overshoots the mark; for, instead of the glumes of the ear being intermediate in length between glumes normal to the ear and the tassel, the glumes of the tunicate ear much exceed normal tassel glumes, and the tassel glumes are themselves much elongated. Neither do the other abnormalities of *Z. tunicata* appear to be typical metaphanic variations, since many of them are repetitious and accentuations rather than intermediate expressions.

There is a sense in which homœotic as well as metaphanic variations may be viewed as a loss or partial loss of differentiation. In typical metaphanic variations the normal specialization of parts is replaced by organs of intermediate form that may be taken to represent an unspecialized ancestral condition. In homœosis instead of the normal specialization certain cells exhibit the characters and potentialities of cells belonging to an entirely different part of the individual. In either instance this loss of specialization or the more or less complete return of cells to an earlier and less specialized condition may be viewed as a reversion.

After concluding that *Z. ramosa* and *Z. tunicata* may be classed as reversions, it is still possible to look upon them as mutations. Both are wide departures from the parent type, and the evidence is that both attained this departure at one step, so far as visible variations are concerned.

With *Z. ramosa* the case is simple. It is a recessive mutation and the dominance of normal maize is complete. The inheritance of *Z. tunicata* is somewhat more complicated. If the interpretation advanced above is correct, and the homozygous form is practically sterile and the common forms heterozygous, *Z. tunicata* would constitute a dominant mutation in which the dominance is imperfect.

The characters separating the two mutations are sharply contrasted. In *Z. ramosa* there is no tendency for the glumes to be elongated more than in normal maize. In tunicate maize the branches of the tassel are no more numerous than in normal maize; and, although the spikelets are much enlarged, the transition from branches to pairs of spikelets is as abrupt as in normal maize. When ears develop on full-tunicate plants, there may be branches near the base of the ear, but these are not at all

homologous to the branches of *Z. ramosa*. The branches in the ear of *Z. tunicata* are proliferated spikelets, while the branches of a *ramosa* ear are, like the branches of the tassel, divisions of the main axis with no evidence of a subtending bract.

DESCRIPTION OF THE HYBRID

The cross between *Z. tunicata* and *Z. ramosa* was made at Lanham, Md., in 1914. The female parent was a plant of *Z. ramosa* grown from seed supplied the Department of Agriculture by Dr. Gernert. The male parent was a plant of a tunicate strain developed by Mr. H. J. Sconce. The parent ear was what is here designated as half tunicate.

Nine first-generation plants of this cross were grown at Chula Vista, Cal., in 1915. Of these, four were tunicate and five normal, indicating the heterozygous nature of the half-tunicate parent plant. The tunicate plants were all half tunicate, and no trace of the *ramosa* characters could be seen.

Five self-pollinated first-generation ears were selected for planting in 1916. Three of these ears were tunicate and two normal. The three tunicate ears all showed about the same development of the tunicate character. The seeds were all well covered by the glumes, but the longest glumes did not exceed 30 mm.

The second generation was grown in 1916 at Lanham, Md. Four hundred and eight plants matured, 326 from the three tunicate ears, and 82 from the two nontunicate ears of the first generation.

The progeny of the nontunicate or normal F_1 plants may be dismissed with the statement that the F_2 plants showed segregation into normal and *ramosa* in the ratio of 3 to 1, the numbers being 65 normal and 17 *ramosa*. In none of these plants was there evidence of tunicate characters.

The first impression to be gained from the descendants of the tunicate plants as they came into flower was that there was a completely heterogeneous mixture of the characters of the two parents, together with many new monstrosities. On closer examination it soon became evident that there was one clearly defined group of plants having all the characters of normal maize. It was also possible to distinguish many plants with characteristic *tunicata* or *ramosa* tassels. Among the latter types, however, there were many intermediates, and in addition there was an entirely new type of inflorescence. In this new type the branching habit was developed to a grotesque extreme. As soon as branches formed these again branched. This division continued until the end of the growing season when the tissue was still in an embryonic condition, and nothing resembling floral or foliar organs was formed. The result was a white succulent mass (Pl. 17, 18). This peculiar formation occurred in both lateral and terminal inflorescences, though it was much more common in the former, and in terminal inflorescences it was usually confined to the basal branches.

This abnormality is similar, if not identical, with an abnormality discovered by Blaringhem (1907) in a strain of *Z. tunicata* and termed by him "cauliflower." To judge from Blaringhem's description and plates, the chief differences between his specimens and ours—and these may be only of degree—are that in his examples the disturbance did not extend to the entire inflorescence, and the ultimate ramification terminated in microscopic floral organs, while in ours no sign of floral organs was developed.

Before an examination of the pistillate inflorescences was possible the growing plants were numbered and classified with respect to the character of the tassel. The classification was made on the general appearance of the tassel, and the following classes were recognized: Normal, half tunicate, full tunicate, *ramosa*, and *tunicata-ramosa*, the last class comprising those plants in which both *tunicata* and *ramosa* characters could be recognized, and frequently with more or less tendency to "cauliflower."

There was no occasion for doubt regarding the plants referred to the normal class. The presence of the *tunicata* character was also unmistakable, but the distinction between half and full tunicate was not always easy to make. In the whole field there were three plants recorded as intermediate between half and full tunicate. The plants referred to *ramosa* formed a fairly distinct class, though it was evident that in many of the plants the tassels were more dense with longer glumes and more nearly pendent than was normal to pure *ramosa*, suggesting the presence of *tunicata* characters. There was, thus, some intergradation between the *ramosa* plants and those classed as *tunicata-ramosa*. This uncertainty was dispelled when the ears were later examined, the *ramosa* and *tunicata-ramosa* classes proving to be completely discontinuous.

GAMETIC COMPOSITION

The numbers in which the various classes of plants occur are capable of explanation by the assumption of a comparatively simple gametic composition. The terminology here used is to assign a letter to the dominant member of each allelomorph and the same letter, primed, to the recessive member. To those who are accustomed to the presence and absence method of notation it will only be necessary to look upon the primed letters as the absence of the factor, usually designated by a lower-case letter. The custom followed by many workers of assigning the unmodified letter to the factor as it exists in the wild or unmutated form is impracticable in agricultural plants. Since we have not this base line, the unmodified letter is assigned to the dominant member.

On this basis, beginning with the dominant form, full-tunicate plants may be assigned the gametic composition $TTRR$, T representing the tunicate factor and R the dominant allelomorph to the *ramosa* factor. *Ramosa*, the other parent, would then be $T'T'R'R'$. Ordinary maize with

respect to these characters would be *T'T'RR*. Half-tunicate plants, such as the male parent of the hybrid, would be *TT'RR*. In a cross between such a plant and *ramosa* the first generation would consist of two kinds of plants, *TT'RR'* and *T'T'RR'*. Since all contain *R*, which is dominant, none would be *ramosa*. One half being heterozygous, for *T* would be half-tunicate, the other half would be nontunicate or normal, being homozygous for *T'*. Since the tunicate plants of the first generation are heterozygous for both *T* and *R*, the selfing of such individuals would give all possible combinations (Table I).

TABLE I.—*Gametic composition of* the *hybrid between Zea ramosa and Zea tunicata*

PARENTS	*Zea tunicata.*	*Zea ramosa.*
P_1 Gametes	*TR T'R*	*T'R'*
F_1 Zygotes	Half tunicate	Normal
Gametes	*TR, TR', T'R, T'R'*	*T'R, T'R'*

F_2

	TR	*TR'*	*T'R*	*T'R'*
TR	*TR* *TR* Full tunicate	*TR'* *TR* Full tunicate	*T'R* *TR* Half tunicate	*T'R'* *TR* Half tunicate
TR'	*TR* *TR'* Full tunicate	*TR'* *TR'* *Tunicata-ramosa*	*T'R* *TR'* Half tunicate	*T'R'* *TR'* *Tunicata-ramosa*
T'R	*TR* *T'R* Half tunicate	*TR'* *T'R* Half tunicate	*T'R* *T'R* Normal	*T'R'* *T'R* Normal
T'R'	*TR* *T'R'* Half tunicate	*TR'* *T'R'* *Tunicata-ramosa*	*T'R* *T'R'* Normal	*T'R'* *T'R'* *Ramosa*

	T'R	*T'R'*
T'R...	*T'R* *T'R* Normal	*T'R'* *T'R* Normal
T'R'...	*T'R* *T'R'* Normal	*T'R'* *T'R'* *Ramosa*

From the above hypothesis and the behavior of the first generation, we should assume that in F_2 all plants homozygous for *R'* would be *ramosa*. All plants either heterozygous or homozygous for *R* and homozygous for *T'* would be normal. Those heterozygous for *T* and with at least one *R* would be half tunicate. Those homozygous for *T* and with at least one *R* would be full tunicate. Since the above conditions are not mutually exclusive, there would be combinations calling for the plants to be *ramosa* and at the same time either full or half tunicate.

There were 326 second-generation plants from the *Z. tunicata* first-generation ears. The observed number compared with the number expected in accordance with the gametic composition assumed above are given in Table II.

TABLE II.—*Comparison of observed and expected ratios of the different classes of plants*

Number expected out of each 16.	Gametic composition.	Characters of plant.	Expected number.	Observed number.
1	$T'T'RR$	Normal	61.2	64
2	$T'T'RR'$	do		
2	$TT'RR$	Half tunicate	122.0	121
4	$TT'RR'$	do		
1	$TTRR$	Full tunicate	61.2	61
2	$TTRR'$	do		
1	$TTR'R'$	*Tunicata-ramosa*	61.2	64
2	$TT'R'R'$	do		
1	$T'T'R'R'$	*Ramosa*	20.4	16
Total 16			326.0	326

When the character of the ear was considered, all the groups, with the exception of half and full tunicate, were perfectly distinct, with no doubtful individuals.

CORRELATIONS BETWEEN TYPE OF PISTILLATE AND STAMINATE INFLORESCENCES

The degree of correlation between the characters of the staminate and pistillate inflorescences may be judged from an examination of Table III.

TABLE III.—*Characters of the staminate and pistillate inflorescences of F_2 plants of Zea ramosa × Zea tunicata hybrid*

Character of pistillate inflorescence.	Character of staminate inflorescence.						
	Normal.	Half tunicate, glumes 12–24 mm.	Full tunicate, glumes above 25 mm.	*Ramosa.*	*Tunicata-ramosa.*	Staminate inflorescences destroyed.	Total.
Normal glumes, 5 mm.	64						64
Half tunicate, glumes 10 to 44 mm.		120	3				123
Full tunicate, glumes above 45 mm. or earless		4	49			6	59
Ramosa				16			16
Tunicata-ramosa				37	23	4	64
Total							326

NORMAL PLANTS.—It will be seen that among normal plants the correlation is perfect. All plants classed as normal by the tassels proved to have normal ears, and vice versa. The number of normal plants was 64; the expected number 61.2.

HALF-TUNICATE PLANTS.—As previously stated, the distinction between half- and full-tunicate plants is not sharp. If one relies on the gen-

eral appearance, there is seldom any doubt regarding the class to which a plant should be referred; but when the differences are formulated, there is some overlapping. The most obvious tassel characters are the long glumes, the pendent tassel, and the presence of pistillate flowers. Of these, the length of glumes appeared to be the most significant, and was the only one systematically recorded. When the glumes are over 25 mm. long, the tassel in most instances obviously belongs to the full-tunicate class. In classifying the ears, the length of the glumes is also the best character. The dividing line here falls on 45 mm.

There were but three half-tunicate plants in which the length of the staminate glumes exceeded 24 mm. There was, however, no perceptible correlation between the length of glumes in the male and female inflorescences among the half-tunicate plants. One hundred and twenty-three plants were referred to this class. The expected was 122. The nearest approach to an intermediate between half-tunicate and normal plants is shown in Plate 19.

FULL-TUNICATE PLANTS.—Fifty-three plants were classed as full tunicate. Of these, 12 produced no ear. Of the remainder, all but 4 had staminate glumes at least 25 mm. long. There is, then, almost a perfect correlation between the type of tassel and the type of the ear; but here again there is no correlation inside the group.

Even the group of plants that were earless did not differ from the plants with ears with respect to the length of the staminate glumes.

There were, in addition, 6 full-tunicate plants with tassels destroyed through accident, making a total of 59 plants in this class. The expected number was 61.2.

RAMOSA PLANTS.—Sixteen plants with pure *ramosa* ears all had *ramosa* tassels. The expected number was 20.4.

TUNICATA-RAMOSA PLANTS.—There were 60 plants that exhibited both *ramosa* and *tunicata* characters. Of these, 23 exhibited the characters of both parents in the tassel as well as the ear. The remaining 37 plants all had *ramosa* tassels in which no tunicate characters were obvious (Pl. 20, *A*).

Of the 23 plants which exhibited both *ramosa* and *tunicata* characters in the tassel, 19 had cauliflower ears (Pl. 20, *B*), 3 showed mixtures of cauliflower and tunicate tendencies, and 1 produced no ear.

In the 37 plants which showed no tunicate character in the tassel, 2 produced cauliflower ears, 11 showed mixtures of cauliflower and tunicate, and 24 were both branched and tunicate without cauliflower (Pl. 21).

In addition to the above, there were 4 plants in which the tassels were accidentally destroyed, making a total of 64 plants in the group. The expected number was 61.2. The nature of the plants combining the characters of both parents is shown in Table IV.

TABLE IV.—*Characters of staminate and pistillate inflorescences of the tunicata-ramosa group of F_2 plants of Zea ramosa × Zea tunicata hybrid*

Character of pistillate inflorescence.	Character of staminate inflorescence.				
	Ramosa.	Without cauliflower.	Partial cauliflower.	Completely cauliflower.	Total.
Ramosa					
Without cauliflower	24				24
Partial cauliflower	11		3		14
Completely cauliflower	2		19		21
Aborted			1		1
Total	37		23		60

If the second-generation plants are examined for each of the parental types separately, there is seen to have been a simple 1 to 3 segregation in both instances. One-fourth of the total number of plants are *ramosa* and three-fourths non-*ramosa* (observed, 79 to 247; expected, 81.5 to 244.5). One-fourth are nontunicate and three-fourths tunicate (observed, 80 to 246; expected, 81.5 to 244.5). The distinction between half and full tunicate could not be made when these characters were combined with the *ramosa* character. The various combinations of parental characters, occurring as they do in the normal dihybrid ratios, show that the *tunicata* and *ramosa* characters are not genetically correlated.

In addition to the notes on the inflorescences, the height of each plant was recorded. From these measurements it develops that there were consistent differences in the height of the segregated groups. The averages are given below:

Type of plant.	Height (cm).
Normal	221±2.7
Ramosa	195±2.2
Half tunicate	195±2.4
Tunicata-ramosa	191±2.6
Full tunicate	171±2.7

These differences in height can hardly be explained as differences in vigor due to different degrees of heterozygosity of the characters concerned, since the *ramosa* and half-tunicate groups, which are of the same and an intermediate height, are at once the least and most heterozygous of all the groups.

ORIGIN AND SIGNIFICANCE OF CAULIFLOWER INFLORESCENCE

In our experiments the appearance of cauliflower in the inflorescence seems definitely confined to plants in which both the *tunicata* and *ramosa* characters are, as it were, endeavoring to come into expression—that is,

to the plants homozygous for the recessive *ramosa* character and either homozygous or heterozygous for the dominant tunicate character.

The intimate relation between the *ramosa* characters and the cauliflower type of inflorescence may have a bearing on the appearance of cauliflower in Blaringhem's strain of *Z. tunicata*. If the two abnormalities are really of the same nature, the possibility is suggested that in Blaringhem's strain there was a re-occurrence of the *ramosa* mutation. Blaringhem had but few plants of this strain under observation; hence, the absence of pure *ramosa* plants would not be remarkable. In the course of our experiments hundreds of plants of *Z. tunicata* have been examined and their abnormalities studied, but nothing resembling the cauliflower type of inflorescence has been found. In the hybrid under discussion, cauliflower is more definitely associated with the *ramosa* than with the *tunicata* characters. The phenomenon itself appears as an accentuation of the branched habit, and while plants with cauliflower ears occurred without exhibiting *tunicata* characters in the tassel, the *ramosa* characters are in all instances fully expressed.

In making this cross between these two variations from normal maize, each of which may be looked upon as a reversion, the hope was entertained that their combination might bring to light still other ancestral characters and help to give us a slightly more definite conception of the ancestors of maize. In this hope of securing direct evidence we were disappointed. When the two characters are forced to come into expression in the same individual, the result is either a mixture of the two characters or a sterile monstrosity which by no stretch of the imagination can be regarded as an ancestral condition.

From the fact that *Z. tunicata* is a dominant variation Blaringhem (1907) concludes that it is a new or progressive mutation without significance in the study of the ancestry of maize. Since in every particular by which *Z. tunicata* departs from normal maize, it does so by replacing the specialized characters of maize with characters common to practically all other grasses, to place so much emphasis on the dominance of the character seems unreasonable.

The phylogenetic bearing of *Z. ramosa* is less obvious, but even here it seems not unwarranted to consider the variation in the nature of a reversion. The partial incompatibility of the two variations may be explained on the assumption that they represent the recurrence of characters from two widely separated ancestors.

SUMMARY

Z. ramosa and *Z. tunicata* are looked upon as mutative reversions, the one recessive, the other dominant, as compared with normal maize. The result of crossing these two mutants has been to show that both behave as independent Mendelian units. In the second generation there appears (1) normal maize showing none of the characters of either muta-

tion, (2) the recurrence of both parental types in an apparently pure form, and (3) plants combining the characters of both the mutations. In the last group normal expression is inhibited, and the result is frequently the appearance of a totally different type of inflorescence called "cauliflower," which is sterile, the character being abnormal to the extent that the tissue remains in an embryonic condition, the result being a much-branched, white, succulent mass.

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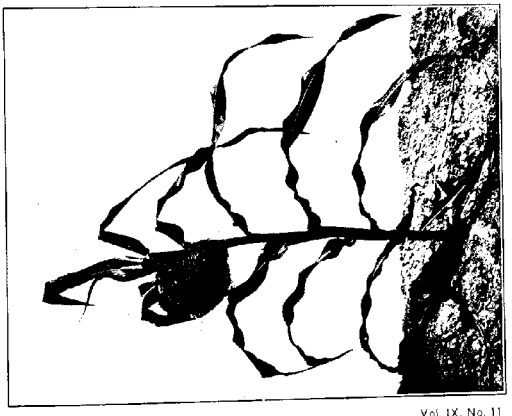
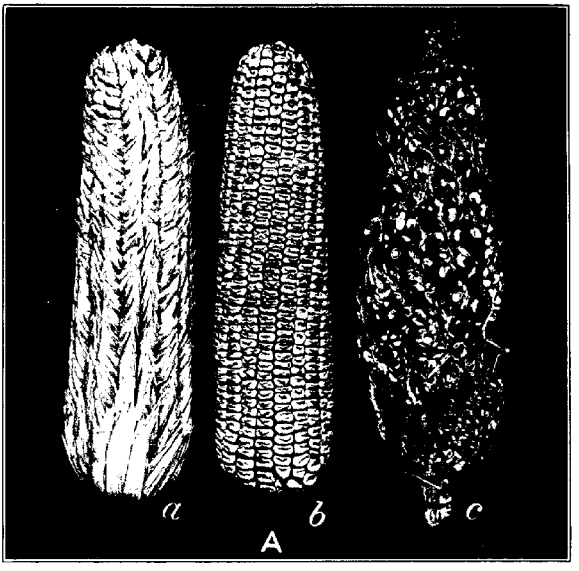
PLATE 13

Zea tunicata:

A.—Plant of full-tunicate type.
B.—Plant of half-tunicate type.




a
b
c
A

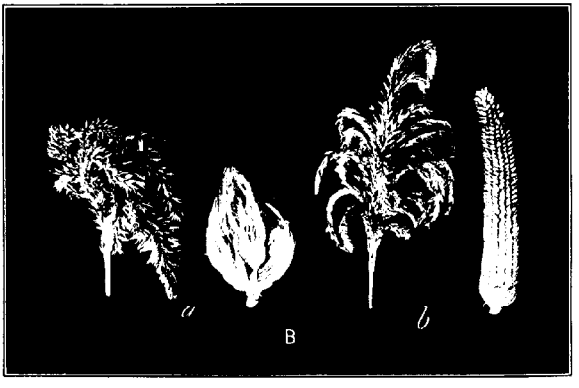

a
B
b

PLATE 14

A.—Pistillate inflorescence of maize. *a*, *Zea tunicata:* Half-tunicate type. *b*, Normal maize. *c*, *Zea ramosa*.

B.—*Zea tunicata:* Terminal and lateral inflorescence. *a*, Full-tunicate type; *b*, half-tunicate type.

PLATE 15

Zea tunicata:

Sterile ear of full-tunicate plant.

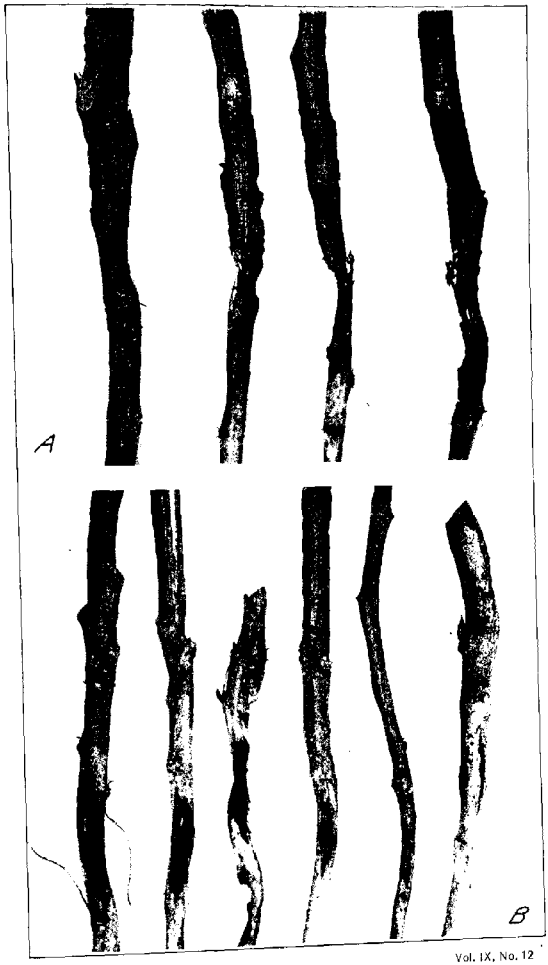
PLATE 16

Zea ramosa:

Staminate and pistillate inflorescence.

PLATE 17

"Cauliflower" lateral inflorescence borne on F_2 plants of *Zea ramosa* × *Zea tunicata* hybrid.


A
B

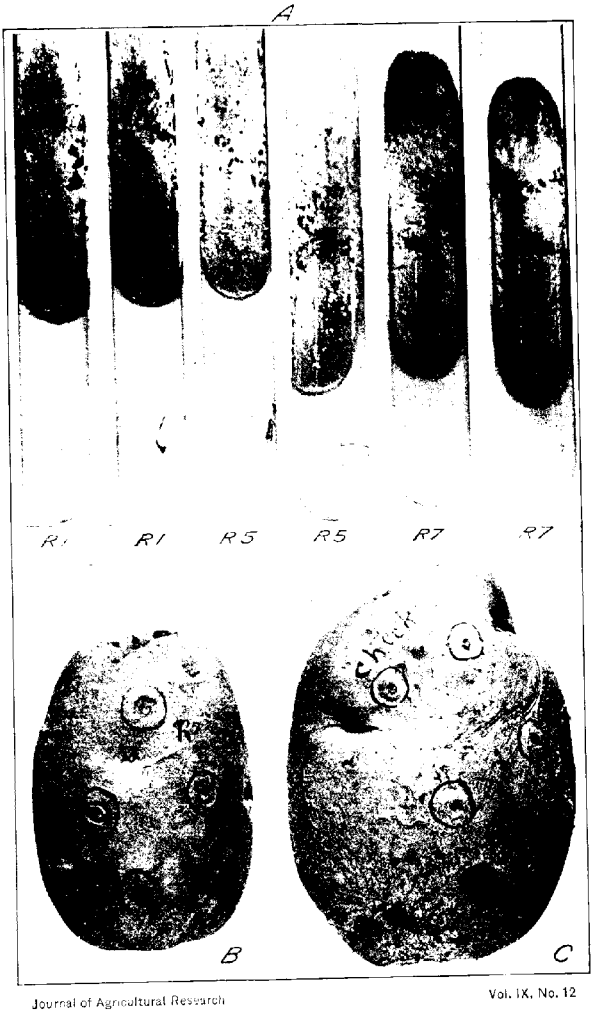

A
R1
R1
R5
R5
R7
R7
B
C

PLATE 26

A.—Reaction on corn-meal agar of R_5, as compared with the other strains. Cultures were made on November 8, 1916, and were photographed on November 27, 1916. The two test tubes to the left show the character of the growth of strain R_1, the next two those of R_5, and the two test tubes to the right those of R_7.

B.—A potato tuber, illustrating the results of inoculation with strain R_5. The inoculation was made on November 5, 1916, and the photograph taken on November 15, 1916.

C.—An injured potato tuber (control). Photographed on November 15, 1916.

A FORM OF POTATO DISEASE PRODUCED BY RHIZOCTONIA

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What appeared to be an undescribed type of potato-tuber disease was observed a few years ago in southern Maine for the first time. Material from this region has been available for study during the present winter (1917). While there is no reason to think that it does not occur in other parts of the State, the writer has not seen any noticeable cases of this trouble in northern Maine, although all of the summer of 1916 was spent in the study of potato (*Solanum tuberosum*) diseases in Aroostook County.

This type of trouble was first recorded in Maine by Morse and Shapovalov.[2] Moreover, after examining a considerable number of potatoes received from various parts of the country as illustrating different types of potato-scab, they were led to make the following statement relative to this form of potato-tuber injury mentioned above:

> A critical examination of potatoes from a large number of sources, including those from other States, has convinced the writers that it is fairly common.

While several authors have mentioned scabbing, pitting, cracking, and ulcer formation more or less in association with Rhizoctonia, so far as the writer has been able to ascertain, with the exception of the publication mentioned above, no other references have been made in the literature to this type of scabbing or pitting, or, as the writer has chosen to call it, "dry core" of the potato tuber. Probably one of the nearest references to this type of trouble was made by Rolfs[3] in his description of an experiment with Rhizoctonia. He placed small amounts of hyphæ from pure cultures of the fungus on the surface of small growing potatoes and covered them with sterilized grafting wax. Fourteen days afterward two of these potatoes were examined, and a number of brown spots were found on the inoculated surfaces. Microscopic examination showed that the hyphæ had entered the lenticels and produced small ruptures in the skin. Further on he states that all of the inoculated tubers developed rough surfaces and cracks, but nothing was mentioned that would lead

[1] Thanks are due to Dr. W. J. Morse for materials and for valuable suggestions in formulating this paper.

[2] Morse, W. J., and Shapovalov, Michael. The Rhizoctonia disease of potatoes. Maine Agr. Exp. Sta. Bul. 230, p. 206. 1914.

[3] Rolfs, F. M. Potato failures. A preliminary report. Colo. Agr. Exp. Sta. Bul. 70, p. 11. 1902.

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Key No. Me.-10

one to believe that anything similar to our type of injury was developed. In a later publication[1] he makes this statement:

> European investigation long ago attributed the pitting or scabbing of tubers to the attacks of *Rhizoctonia*. Our experiments and observations also show that its attacks on growing tubers frequently produce deep ulcers. Most of our scab is due to the attacks of this fungus.

Other authors at about this same time observed Rhizoctonia hyphæ associated with the so-called scab ulcers, but were of the opinion that this fungus had nothing to do with their formation. However, in no case has the writer been able to find either photographs, drawings, or descriptions that give any adequate idea as to just what is meant by a scab ulcer.

Without a doubt this form of trouble has escaped the critical attention of pathologists for several years, owing to the fact that in some ways, upon superficial examination, it may frequently appear somewhat similar to the common scab of potatoes. However, upon careful comparison no one can have the slightest doubt but that this dry core or pitting is entirely different from the form of injury produced by *Oospora scabies* Thaxter, later considered by Lutman and Cunningham as identical with *Actinomyces chromogenus* Gasperini.

In greenhouse experiments Morse and Shapovalov[2] observed that this pitting was closely associated with Rhizoctonia. They found Rhizoctonia filaments within these pits and that infection apparently originated at the lenticels. However, their conclusions were based entirely upon general observations and upon the fact that the filaments of the fungus were constantly associated with all stages of the development of the diseased areas. No critical study was made of the relationship of the supposed parasite to the host tissues.

It is the purpose of this paper to describe this form of disease more fully and to present evidence which tends to show that *Rhizoctonia solani* Kühn (*Corticium vagum* B. and C.) is largely, if not entirely, responsible for this type of injury.

There are two phases of this disease that should be noted. First, the stage that on superficial examination might be mistaken for common scab. Seopnd, a stage showing a canal formation which might be confused with the injury caused by the wireworm. The first stage is most generally noticeable where the infection is less than 3 mm. in diameter. However, there are exceptions to this.

The fungus enters at the lenticels and works its way down into the tuber without much external disturbance. The original outer cortex, being left more or less intact, forms a roof over the diseased area. The definite boundary and dark-brown color of the area suggest a form of scab. The interior granular mass of hyphæ, broken-down cells, and

[1] Rolfs, F. M. Potato failures. A second report. Colo. Agr. Exp. Sta. Bul. 91, p. 11. 1904.
[2] Morse, W. J., and Shapovalov, Michael. Loc. cit.

starch grains all remain in position, forming a dry "plug" and suggesting the name "dry core."

The second phase of this type of injury is most often found in the older stages in the progress of the disease or where the infected area reaches a diameter greater than 3 mm. This stage is most likely to be found when the potato tubers have reached maturity and the disease has run its course, or after the tubers have been stored. Owing to a drying out and shrinkage of tissues, a pit or canal is formed in the center of the affected area. Doubtless in harvesting and storing the tubers this pitting is furthered by parts of this granular material being shaken out or loosened. There is no doubt that the greater part of this pitting, particularly in cases where the disease has penetrated deeply into the flesh, has been attributed in Maine to the work of the wireworm. However, close observation even by the layman will readily show a great difference between these two types of injury.

The diseased areas are approximately circular in outline and at the surface vary in size from that of a lenticel to 6 or 7 mm. in diameter. They usually extend into the flesh of the tuber to a depth equal to or somewhat greater than the diameter. The dry core or pit thus formed gradually tapers off, forming a somewhat rounded end, very seldom becoming pointed. The majority of these pits are proportioned and shaped quite like a thimble, but some of them have a tendency to become longer and more slender. In such cases a dry, roughly cylindrical core may penetrate the flesh of the tuber for some distance. A casual observer might readily attribute this form of injury to insect attacks. In a few cases the pit has taken a more or less horizontal direction in reference to the surface of the tuber, and, as shown in Plate 27, *F*, two of these pits have joined together some little distance below the surface of the tuber.

Infection takes place in lenticels. Even from the very earliest stages the infected areas become slightly darker and sunken from the surrounding tissues. The mycelium of the fungus seems to travel equally in all directions; thus, as time passes, the infected area becomes a larger and larger circle dark brown in color. Surrounding the mature pit there is a very definite line of demarcation separating the diseased tissue from the healthy. In fact, this line is so definitely laid down that one has little or no difficulty in inserting the point of a knife and lifting out the whole core, leaving a clear-cut cavity in the healthy flesh of the tuber. By boiling tubers affected in this manner, the majority of the cores will come out clean with the peeling when it is removed. Plate 28, *E–I*, shows a group of these cores that came out with the peeling after the tuber has been boiled for 20 minutes. It will be seen (Pl. 29, *A*) that this division line is formed by three or four layers of compact suberized cells that have been laid down by the potato to prevent further penetration of its tissues by the fungal hyphæ. This leather-like lining of the

pit, once thoroughly established, effectively stops further progress of the fungus. No fungal filaments have ever been found penetrating this lining, and none have been found in the healthy tissues surrounding any of these pits. The evidence shows that the size of the pit is determined by the rate of suberization of cells sufficiently far enough in advance to effectively block further progress of the fungus. From very earliest infection, when the diseased area is scarcely more than an enlarged lenticel, the host cells always show more or less suberization three or four cells in advance of the deepest situated fungal filament. This being uniformly the case, together with the fact that the cells seem to die and lose their contents some distance ahead of the fungus, has suggested the possibility of a toxic substance being secreted by the hyphæ.

Microtome sections varying from 8 to 15 μ in thickness have been made from all stages of the "dry core" from the earliest, showing only the infected lenticel, to the age when the diseased area has ceased to enlarge, some as great as 6 mm. in diameter. In every case Rhizoctonia mycelium has been found. These infected areas are remarkably free from secondary fungi. In characteristic cases there is no histological evidence whatever of the presence of any other fungus. This, coupled with the fact that pure cultures of Rhizoctonia have been obtained repeatedly from these infections, seems to present a very strong case against *Rhizoctonia solani* Kühn (*Corticium vagum* B. and C.).

What appeared on the surface of a tuber as an enlarged lenticel is shown in section in Plate 30, *B*. After sectioning and staining this material, it was a matter of surprise to find Rhizoctonia hyphæ so readily, and in all sections made of this area. It will be seen from the illustrations that the host cells have already begun to suberize four cells in advance of the fungal hyphæ. No doubt this displacement and ragged edges of the normal corky cells of the cortex has been emphasized on account of their being washed and torn out in the process of fixing and cutting. However, it is believed that this will not prove misleading to anyone who has had experience in working with materials of this kind. The gradual progress of the fungus and the disintegration of the host cells, in what might be termed the second step, is shown in figure *C* (Pl. 30). This affected area was slightly over 1 mm. in diameter. By comparison with figure *A* (Pl. 30), which is a section of a normal portion of this same tuber, the results produced by the invading fungus may readily be seen. As the infection spreads and the fungal filaments become more and more abundant, this disintegration process goes on until the interior of the core is converted into a mass of broken-down cells, hyphæ, and free starch, thus giving an appearance, when magnified, similar to Plate 30, *D*. The hyphæ in this case will be seen to be of the sclerotia-forming type, as compared with the earlier infection stages or with the pure-culture hyphæ of figure *F* (Pl. 30). In a few cases the writer had the good fortune to get sections in which the outer cortex had remained more or less

intact, thus preventing the loss of the granular mass inside the core. Sclerotia-forming hyphæ were very abundant and in some instances, such as figured in Plate 30, *D*, cells more or less broken down were completely filled and apparently held in shape by the compact mass of mycelium. The mycelium of this character seemed to adhere to or follow the cell walls to a great extent.

The writer has seen little evidence of actual cell-wall penetration by the hyphæ. While Plate 29, *B*, seems to show that the fungal filaments can penetrate the walls, yet from the numerous sections made of diseased tissue practically no other cases could be found, although diligent search was made. The host cells are always found to have been killed, their walls suberized and more or less collapsed, quite some distance in advance of the fungal hyphæ. A falling apart and a separation of the cell walls suggest some kind of action upon the middle lamellæ. All of this action, taken together with the natural mechanical breakdown due to drying out and shrinkage, soon converts the interior of the core into a dry granular mass partially held together by the mycelium of the fungus.

Drayton,[1] in his study of Rhizoctonia lesions upon the potato stem, says:

> Individual hyphae were found running longitudinally and sometimes obliquely in the cells of these tissues and in the intercellular spaces.

This statement is made with reference to the vascular bundles and pith of the stem, and, although he does not actually say that cell-wall penetration takes place, yet one might infer that this does occur. It is believed, however, that this does not necessarily conflict with the present findings. Within the tuber the hyphæ find quite a different situation and seemingly have considerable difficulty in penetrating the cell walls.

SUMMARY

(1) Direct mention of this form of potato injury was first made in Maine. If reference elsewhere has been made to it, the descriptions were not adequate to warrant connection with this form of injury.

(2) Two phases of the injury are worthy of notice: One whose external appearance somewhat resembles scab and which extends as a dry core into the flesh of the tuber; another in which the shrinkage of tissues has formed a pit or canal in the center of the infected area, frequently suggesting wireworm injury.

(3) Careful histological studies of all stages in the progress of the injury invariably show the presence of Rhizoctonia hyphæ.

(4) Pure cultures of Rhizoctonia have repeatedly been obtained from the interior parts of the diseased areas.

(5) Evidence shows that the host cells die and lose their contents, and the walls suberize and are more or less broken down several cells in

[1] Drayton, F. L. The Rhizoctonia lesions on potato stems. *In* Phytopathology, v. 5, no. 1, p. 61. 1915.

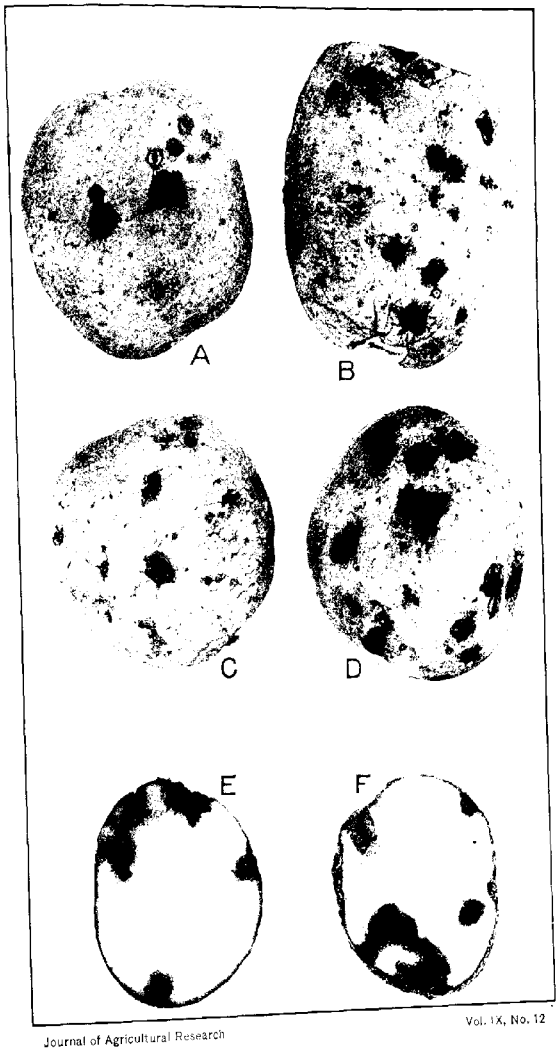
advance of the fungal filaments. This might lead one to suspect that part of the action is due to a toxin that is secreted by the fungus.

(6) Actual cell-wall penetration by the Rhizoctonia hyphæ apparently may occur, but this seems to be the exception rather than the rule.

PLATE 27

A-D.—Various stages of the "dry core" of potato tubers.

E-F.—Cross sections of a tuber badly affected with Rhizoctonia. Figure F shows two of the cores joined together. Compare with figure E of Plate 28, which illustrates a similar core taken bodily out of a boiled potato.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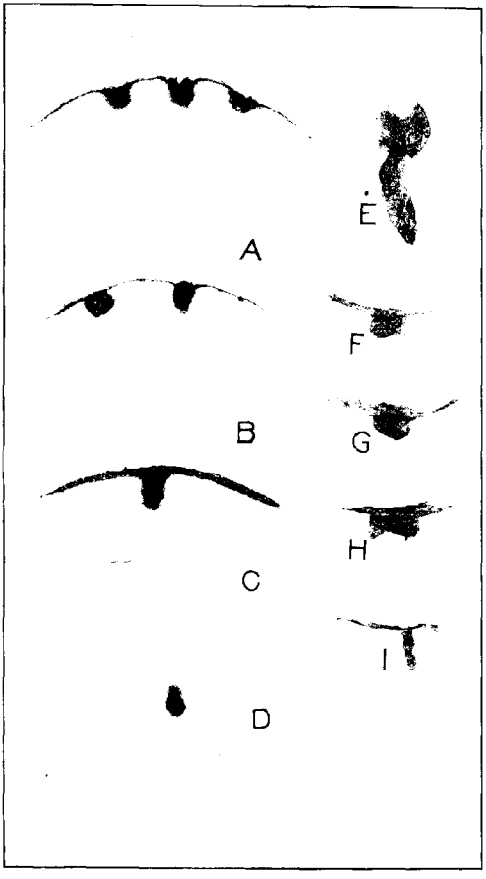
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PLATE 28

A-C.—A longitudinal section through some of the cores of affected potato tubers. Note the dry, granular mass still inside of the darkly outlined pits.

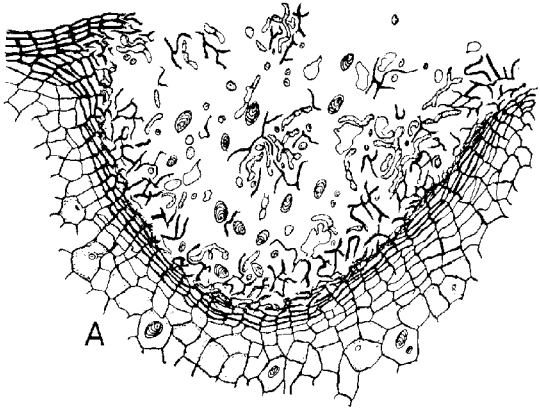
D.—Transverse section of a core.

E-I.—"Dry cores," giving a good idea of their size and shape. These were lifted out of a boiled potato.

PLATE 29

A.—A pit slightly over 2 mm. in diameter, showing broken down cells, free starch, and fungal hyphæ. Note the layer of suberized cells arising from the outer cortex and forming a lining to the pit.

B.—Fungal hyphæ apparently penetrating the cell walls.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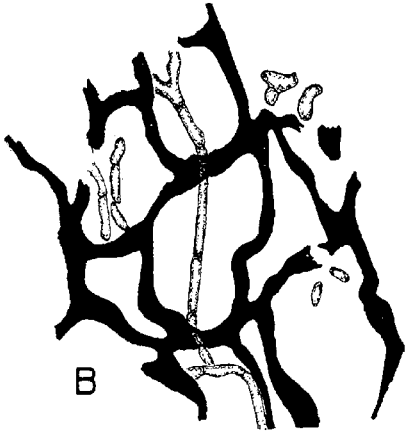
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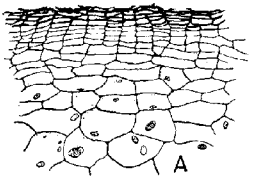
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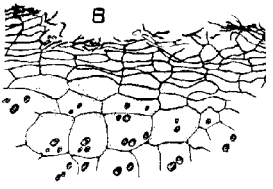
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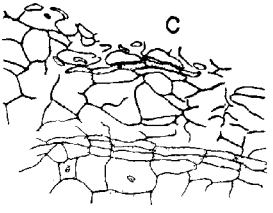
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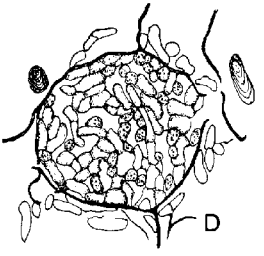
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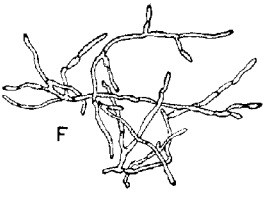
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PLATE 30

A.—The normal cortex and inner tissues of a potato tuber.

B.—An enlarged lenticel, showing a very early stage of infection.

C.—Fungal filaments and broken down cells in an infected lenticel slightly over 1 mm. in diameter.

D.—A host cell highly magnified showing the interior filled with fungal hyphæ.

E.—The granular contents of one of the pits as it appears under the microscope.

F.—Rhizoctonia hyphæ from a pure culture isolated from the interior part of a dry core.

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ERRATA

Page 110, line 6 from bottom, "$\frac{-7.26a}{A}$" should read "$\frac{-0.26a}{A}$".

Page 123, line 3, "c" should read "C".

Page 424, line 5 from bottom, "D" should read "E."

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